PATENT
Attorney Docket No. 06854.0024-01

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:	
Masahiro KAJIWARA	Group Art Unit: 1625
Application No.: 10/669,700	Examiner: D. Margaret M. Seaman
Filed: September 25, 2003	
For: UREASE INHIBITORS	Confirmation No.: 6367

Attention: Mail Stop Appeal Brief-Patents
Commissioner for Patents
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Alexandria, VA 22313-1450

APPEAL BRIEF UNDER BOARD RULE § 41.37

In support of the Notice of Appeal filed May 5, 2006, and further to Board Rule 41.37, Appellant presents this brief and enclose herewith a check for the fee of $500.00 required under 37 C.F.R. § 1.17(c).

This Appeal responds to the February 6, 2006, final rejection of claims 9-20.

If any additional fees are required or if the enclosed payment is insufficient, Appellant requests that the required fees be charged to Deposit Account No. 06-0916.

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I. Real Party In Interest

Otsuka Pharmaceutical Co., Ltd. is the real party in interest.

II. Related Appeals And Interferences

There are currently no other appeals or interferences, of which Appellant, Appellant's legal representative, or Assignee are aware, that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. Status Of Claims

Claims 9-20 are currently pending and have been finally rejected by the Examiner. Appellant appeals the rejection of those claims.

Further to 37 C.F.R. § 41.37(c)(1)(viii), the attached Appendix contains a clean copy of the claims.

IV. Status Of Amendments

An Amendment After Final under 37 C.F.R. § 1.116 was filed on June 30, 2006, to propose corrections to clerical errors in claims 17 and 19. To date, the Amendment has not been acted upon by the Office.

V. Summary Of Claimed Subject Matter

The present invention is based on the novel and non-obvious finding that isothiazole compounds according to claimed formula (I) have excellent urease inhibitory activity and an excellent anti-*Helicobacter pylori* ("*H.pylori*") activity. (*E.g.*, Specification, pg. 3, ll. 17-21.) This is significant, because it has recently been made clear that urease, such as urease produced by *H.pylori*, has a close relationship to gastric mucosa injury and gastrointestinal disease, such as chronic gastritis and gastroduodenal ulcer. (*E.g.*, Specification, pg. 1, ll. 9-13.)

In this regard, the first independent claim, claim 9, is directed to a method of treating gastric mucosa injury caused by urease, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound of formula (1). (*E.g.*, Specification, pg. 3, ll. 27 - pg. 4, ll. 4.) The next independent claim, claim 16, is directed to a method of treating gastric mucosa injury caused by *H.pylori*, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound of formula (1). (*E.g.*, Specification, pg. 4, ll. 5-8.) The remainder of the independent claims, claims 17, 18, 19, and 20 are directed, respectively, to methods of treating chronic gastritis caused by urease, gastroduodenal ulcer caused by urease, chronic gastritis caused by *H.pylori*, and treating a gastroduodenal ulcer caused by *H.pylori*. (*E.g.*, Specification, pg. 3, ll. 22 - pg. 4, ll. 8.) In addition, claim 14 is directed to a method of treating gastric mucosa injury caused by urease according to claim 9, the method further comprising administering at least one additional pharmacologically active ingredient. (*E.g.*, Specification, pg. 15, ll. 13-24.)

VI. Grounds Of Rejection

Claims 9-20 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Japanese Patent No. 04077476 to Hirai ("Hirai"). The Examiner has contended that Hirai inherently discloses use of a compound to treat ulcers by inhibiting urease or *H.Pylori* activity. (E.g., February 6, 2006, Office Action, pg. 2-3.) The Examiner has not contended that Hirai expressly discloses methods of treating gastric mucosa injury caused by urease or *H.Pylori*. The Examiner also has not contended that Hirai discloses methods of treating chronic gastritis. The Examiner has also not contended that Hirai discloses methods of treating gastric mucosa injury, where the method comprising administering at least one additional pharmacologically active agent.

VII. Argument

A. Claim Group 1: Claims 9, 10, 12, 13, 15, 16, 18, and 20

1. Summary Of Appellant's Position

The presently appealed rejection under 35 U.S.C. § 102(b) over Hirai (JP04077476) necessarily fails because Hirai at most discloses the treatment of ulcers and does not inherently disclose the claimed methods of treating gastric mucosa injury caused by urease or *H.Pylori*. As made clear by the Federal Circuit's December 2005, Perricone v. Medicis Pharmaceutical Corp. decision, "[t]he issue" pertinent to an inherency-based rejection of a method of treatment claim directed to, for example, treating a specific type of injury "is not... whether [the prior art composition] if applied to [a damaged area] would inherently treat that damage, but whether [the prior art] discloses the application of its composition [to the specified type of damaged area]." 77 USPQ2d 1321, 1328 (Fed. Cir. 2005). Just as not all skin surfaces are skin sunburn surfaces, Perricone at 1328, not all ulcers are caused by urease or *H.Pylori*. Therefore, the treatment of ulcers is not an inherent disclosure of the treatment of gastric mucosa injury caused by urease or *H.Pylori*. For example, the presently claimed methods are not inherent in Hirai's treatment of ulcers or other conditions at least because, in the large number of ulcer patients not having injury caused by urease or *Helicobacter pylori* infection, the use of a compound according to Hirai would not and cannot treat injury caused by urease or *Helicobacter pylori* when neither is present or where neither is the cause of injury.

In particular, after citing Hirai for disclosing the biological properties of certain compounds but without identifying any disclosure providing for the use of these compounds to treat gastric mucosa injury caused by urease or *H.Pylori*, the Examiner alleged that the present claims are inherently taught by Hirai because "[t]he method of treating ulcer in a patient using

the same prior art compound would inherently inhibit urease activity and helicobactor pylori activity of any mechanism. The mechanism of action fails to set a demarcation...." (February 6, 2006, Office Action, pg. 2-3.) Just like *Perricone*, the question, however, is not what are the inherent properties of the compounds if used according to the presently claimed methods. Rather, the relevant question is whether the claimed methods are inherently disclosed by Hirai. They are not.

2. Inherency For A Method Of Treatment Requires That The Claimed Method Is Necessarily And Always Achieved By The Prior Art Method

To establish inherency, the evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference...." Continental Can Co. USA, Inc. v. Monsanto Co., 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) (emphasis added). It is also well settled that "[i]nherency... may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." Id. (internal citations omitted) (emphasis added). That is, the fact that a certain method or result may occur in some circumstances or under limited conditions is not sufficient to establish the inherency of that method or result.

In this regard, the recent Federal Circuit decision in Perricone v. Medicis Pharmaceutical Corp. is particularly relevant. 77 USPQ2d at 1328. At issue in Perricone were, among other things, the inherent anticipation of claims directed to methods of treating or preventing skin sunburn comprising applying to the skin sunburn a composition having certain properties. Id. at 1323-24. The prior art reference had been found by the District Court to disclose a composition having the properties recited in the claim, and to further disclose the composition as "suitable for topical application to the skin or hair." Id. at 1326. The Federal Circuit distilled the inherency

analysis to the question of whether the prior art's "disclosure of 'topical application' satisfies the [claimed] application step [to treat skin sunburn]...." Id. at 1328. The inherent properties of the composition were not at issue, as "[n]ew uses of old products or processes are indeed patentable subject matter." Id. (citations omitted).

In its analysis, the Court explained that for the claimed methods of treating skin sunburn, "[t]he issue is not... whether the [prior art's] lotion if applied to skin sunburn would inherently treat that damage, but whether [the prior art] discloses the application of its composition to skin sunburn." Id. at 1328. The Federal Circuit found that it did not:

> The disclosed use of [the prior art's] lotion, i.e., topical application, does not suggest application of [the prior art's] lotion to skin sunburn. In other words, the district court's inherency analysis goes astray because it assumes what [the prior art] neither disclosed nor rendered inherent. Because [the prior art] does not disclose topical application to skin sunburn, this court reverses the district court's holding that [the prior art] anticipates claims 1-4 and 7 of the '693 patent.

Perricone at 1328.

The Court's holding expressly rejected reasoning that failed to recognize distinctions between the claimed method, which requires "applying to the skin sunburn," and the prior art's disclosed method, which only disclosed application to skin surfaces generally. The distinction is, of course, that "[s]kin sunburn is not analogous to skin surfaces generally." Perricone at 1328. As an illustration of this distinction, Perricone further noted that "the disclosure that a sunburn can be prevented by wearing a hat clearly does not anticipate a claim to the discovery that one can treat an existing sunburn by putting on a hat." Perricone at 1328 (emphasis added).

In contrast to the claims requiring treatment of skin sunburn, the Court affirmed the holding of inherent anticipation for the claim (claim 8) directed more broadly to applying "[a] method for preventing sunburn damage to exposed skin surfaces, comprising topically applying

to said skin surfaces...." Perricone at 1328 (emphasis added). The reasoning finding inherent anticipation of this "prevention" claim was that "[b]ecause all skin surfaces are susceptible to sunburn damage, and because one can only realistically apply a composition to a skin surface when that surface is exposed, [the prior art's] 'topical application' encompasses the application step of claim 8." Perricone at 1328.

Thus, by addressing the issues of what methods are and what methods are not inherently disclosed in a general or non-specific prior art reference, the Federal Circuit further clarified the relevant considerations for evaluating the inherency of a method directed to the use of known compounds. First, the disclosed use of a compound for some broad application, e.g., topical application to the skin, is not an inherent disclosure of using the compound to treat specific types of conditions or areas, e.g., skin sunburn. Perricone at 1328. Second, and in contrast to the first principle, where there is no realistic distinction between the prior art method, e.g., topical application to the skin, and the claimed method, e.g., application to exposed skin, the later claimed method is inherently disclosed. Id.

Appellants further note the Federal Circuit's decisions in Rapoport v. Dement, 254 F.3d 1053 (Fed. Cir. 2001) and Jansen v. Rexall Sundown, Inc., 342 F.3d 1329, 1333-34 (Fed. Cir. 2003), as being pertinent to the issue of inherent anticipation. In Rapoport, an appeal from an interference proceeding before the Board, the count at issue was:

> A method for treatment of sleep apneas comprising administration of a therapeutically effective amount of a Formula I azapirone compound or a pharmaceutically effective acid addition salt thereof to a patient in need of such treatment . . . .

254 F.3d at 1056 (emphasis added). On appeal, the Federal Circuit interpreted the preamble phrase "for treatment of sleep apneas" to refer to sleep apnea treatment per se, not merely treatment of symptoms associated with sleep apnea. Id. at 1059. Specifically, one party in

Rapoport had argued that the count was anticipated by a reference disclosing the use of the claimed compound for treatment of anxiety and breathing difficulty, which are symptoms of sleep apnea, even though the reference did not provide for treatment of sleep apnea itself. Id. at 1061. The Court rejected that argument. While the reference mentioned the possibility of administering the compound to patients suffering from sleep apnea, "[t]here is no disclosure in the [prior art reference that the compound] is administered to patients suffering from sleep apnea with the intent to cure the underlying condition." Id. (emphasis added). Thus, since the claim was interpreted to require that the method be practiced with the intent to achieve the objective stated in the preamble, it was not anticipated by a reference lacking a teaching of treating sleep apnea.

In Jansen v. Rexall Sundown, Inc., the Federal Circuit reaffirmed the holding of Rapoport that a claimed method of treatment is not invalidated by a prior art reference unless that reference provides for practicing the method with the intent to achieve the claimed objective. 342 F.3d 1329, 1333-34 (Fed. Cir. 2003) ("In other words, administering the claimed vitamins in the claimed doses for some purpose other than [the claimed] treating or preventing macrocytic-megaloblastic anemia is not practicing the claimed method....") See also Glaxo Group Ltd. v. Teva Pharma, Inc., 2004 U.S. Dist. LEXIS 16750, at *56-57 (D. Del. 2004).

3. It Is Uncontested That Not All Ulcers Are Caused By Urease Or *H.Pylori*

The fact that not all ulcers are caused by urease or *H.Pylori* is well documented, uncontested, and of particular relevance to this case. It is particularly relevant because, just as the application of a composition to skin surfaces generally was not a disclosure of applying the composition to skin sunburn, Perricone at 1328, the treatment of ulcers generally, including those not associated with or caused by urease or *H.pylori*, is not the disclosure of treating gastric

mucosa injury caused by urease or *H.pylori*. The documented and uncontested facts are that ulcers can be independently caused by excess ATPase activity and irritants, such as non-steroidal anti-inflammatory ("NSAID") drugs like aspirin. Further, it is generally considered that *Helicobacter pylori* is distinct from ATPase activity, and directly injures gastric mucosa or produces inflammatory-causing factor which brings about epithelium cell injuries on gastric mucosa. Thus, as explained further below, based on evidence of record in this case, gastrointestinal ulcers can be caused by a variety of conditions or factors, including:

> 1) infection with *Helicobacter pylori*,
>
> 2) use of non-steroidal anti-inflammatory drugs: NSAIDs (e.g., aspirin),
>
> 3) unusually strong digestive activity with excess secretion of gastric acid, or
>
> 4) others (e.g., stress).

See, e.g., "Reduce Your Risk" brochure from the American Gastroenterological Association and American Pharmacists Association (Attachment A).)

For example, according to Huang, J.Q. et al., Lancent, 359:14-22, 2002 ("Huang") (Attachment B) both *Helicobacter pylori* infection and NSAID use independently and significantly increase the risk of peptic ulcer and ulcer bleeding (Huang, pg. 14, col. 1, Interpretation.) While having *Helicobacter pylori* infection and also taking NSAIDs increased the risk of bleeding ulcers,[1] a substantial portion of NSAID patients had ulcers in the absence of *Helicobacter pylori* infection. (Huang, pg. 14, col. 1, Findings, Interpretation.) Further, the

[1] The possibility that ulcer may be aggravated by both NSAIDs and *Helicobacter pylori* does change the fact that ulcers may be due to independent causes, such as NSAIDs alone in the absence of *Helicobacter pylori* infection, and that the treatment of these ulcers is not (even inherently) a treatment of injury caused by *Helicobacter pylori* infection or urease activity.

contribution of NSAIDs is significant, since peptic ulcer disease was significantly more common in NSAID users irrespective of *Helicobacter pylori* infection. (Huang, pg. 14, col. 1, Findings.)

The fact that use of NSAIDs results in peptic ulcer by a mechanism different from that of *Helicobacter pylori* infection is further shown in Wolfe, M.M. et al.: N. Engl. J. Med., 340:1888-1899, 1888 (1999) (Attachment C) ("Numerous reports have corroborated [that aspirin could cause gastric mucosal damage], and the introduction of more potent agents with an even greater propensity for toxic effects has increased the awareness of NSAID-induced gastroduodestal ulcers....") Wallace, J.L. et al.: Gastroenterology, 119:706-714, 713 (2000) (Attachment D) ("In this study we focused on the suppression of COX activity by NSAIDs as a mechanism of gastric injury. Of course, NSAIDs have other actions unrelated to suppression of COX that contribute to damage. For example, many NSAIDs exert topical irritant effects that can contribute to mucosal injury."), and Langenbach, R. et al.: Cell, 83:483-492, 484 (1995) (Attachment E) ("[W]hile NSAIDs have many beneficial effects, they can also cause adverse effects, the most common of which are gastrointestinal ulceration and nephrotoxicity....").

Thus, all gastrointestinal ulcers are not caused by or associated with urease or *Helicobacter pylori* infection. For this reason, the treatment of an ulcer generally, or more specifically, as one example, an ulcer caused by NSAIDs, would not inherently be treating injury caused by urease or *Helicobacter pylori* infection, which are not present, associated with, or the cause of all ulcers.

4. Hirai Is directed To Treating Ulcers By Reducing Acid Via Proton-Pump Inhibition

Hirai does not teach (expressly or inherently) or suggest a method for treating gastric mucosa injury caused by urease or *Helicobacter pylori* activity. To the contrary, the limited disclosure according to Hiria related to treating ulcers is as follows:

1) "It has been known that 2-[(3,5-dimethyl-4-methoxy-2-pyridyl)methylsulfinil]-5-methoxy-(1H)-benzimidazole (omeprazole) (a compound described in Japan kokai 54-141783) is clinically effective for suppressing gastric acid secretion as antiulcer agent by inhibiting H+, K+-adenosine triphosphatase (H+, K+ -ATP) which is an enzyme relating to the final stage of gastric acid secretion." (See Prior Art on page 4 of the English translation.)

2) "The inventors made earnest studies on synthesis of benzisothiazolone derivatives and their pharmacological activity for years, consequently they discovered that the compounds of this invention have an enzymatic inhibition activity of H+, K+-adenosine triphosphatase over 100 times as much as that of above known omeprazole and become an excellent agent for preventing and treating ulcer, thus came to accomplish this invention." (See Problem to be Solved by the Invention on page 4 of the English translation.)

3) "As described above, the compound (I) of this invention have excellent inhibitory action on H+, K+-ATPase and are useful for the prevention or treatment of gastrointestinal diseases, etc., of mankind, e.g., gastric ulcer, duodenal ulcer, or Zollinger-Ellison syndrome, etc." (See page 39 of English translation.)

Thus, Hirai simply and only discloses a method of preventing or treating gastrointestinal diseases caused by "inhibitory action on H+, K+-ATPase," *i.e.,* proton-pump inhibition (see below), according to administration of the compound (I).

In this regard, many common ulcer treatments are similarly not directed to inhibition of urease or *Helicobacter pylori* activity. For example, the drug omeprazole (cited for comparative H^+, K^+ -ATPase activity in Hirai, Abstract, pg. 4) is known as a "proton-pump" inhibitor due to its H^+, K^+ -ATPase activity, which decreases the amount of acid produced in the stomach. (See entry for "Prilosec®", in Physicians' Desk Reference ("PDR"), pages 633-638, at 637 (2004) (Attachment F). Ulcers and other gastric conditions treated by omeprazole may be caused by other than urease or *Helicobacter pylori* infection, and the use of omeprazole for these conditions thus does not inhibit urease or *Helicobacter pylori* activity or treat injury caused thereby. (See

PDR at 636 (Prilosec® indicated for the treatment of, among other things, active duodenal ulcer, distinct from the treatment of duodenal ulcers associated with *H. pylori* infection).)

Accordingly, the disclosure of a compound (*e.g.*, omeprazole or a compound according to Hirai) to treat ulcers is not necessarily the same as the use of that compound to inhibit urease or *Helicobacter pylori* activity. Indeed, according to the PDR, an antibiotic must be used with omeprazole to treat *Helicobacter pylori* infections associated with ulcers. (*E.g.*, PDR at 636.)

Since Hirai only suggests that their compounds have H^+, K^+ -ATPase proton-pump activity, which are indicated for the treatment of ulcers not necessarily associated with or caused by urease or *Helicobacter pylori*, the use of a compound according to Hirai to treat ulcers would not necessarily inhibit urease or *Helicobacter pylori* or treat injury caused thereby. This is true regardless of whether the compound also has anti-urease or *Helicobacter pylori* activity. Thus, Hirai is silent about, among other things treating gastric mucosa injury, gastrointestinal ulcer, and chronic gastritis caused by urease or *Helicobacter pylori*.

5. There Is No Legal Or Factual Support For The Inherency-Based Rejection

In view of the above, there is no support for the Examiner to maintain that Hirai inherently discloses a method of treating gastric mucosa injury by inhibition of urease or anti-*Helicobacter pylori* activity, as more specifically set forth in the claims. The reference simply does not teach (or even suggest) the method as claimed. Among other things, in the large number of ulcer patients that do not have *Helicobacter pylori* infection or injury caused by urease or *H.pylori*, the use of a compound according to Hirai would not and cannot inherently treat injury caused by urease or *Helicobacter pylori*.

The present facts are analogous to Perricone, and, indeed, even more compelling. In particular, at most, Hirai discloses use of a compound to treat ulcers generally. More precisely,

Hirai discloses the compound with reference to proton pump inhibitors, thereby indicating their compound to be applicable to treat ulcers requiring proton pump inhibition. (E.g., Hirai translation, pg. 4 ("The inventors made earnest studies on synthesis of benzisothiazolone derivatives and their pharmacological activity for years, consequently they discovered that the compounds of this invention have an enzymatic inhibition activity of H+, K+-adenosine triphosphatase over 100 times as much as that of above known omeprazole and become an excellent agent for preventing and treating ulcer, thus came to accomplish this invention"); pg. 39 ("As described above, the compound (I) of this invention have excellent inhibitory action on H+, K+-ATPase of and are useful for the prevention or treatment of gastrointestinal diseases, etc., of mankind, e.g., gastric ulcer, duodenal ulcer, or Zollinger-Ellison syndrome, etc.").) Accordingly, rather than a general treatment, as was the case with the prior art in Perricone, Hirai is specifically directed to ulcer treatment by proton-pump inhibition. Ulcers suitable for treatment by proton-pump inhibition are known, and have been shown by uncontested evidence, to include those with etiologies independent of urease and *H.pylori* infection.

Thus, because Hirai does not teach any method for the treatment of gastric mucosa injury caused by urease or *H.pylori*, Hirai fails to anticipate claims 9, 10, 12, 13, 15, 16, or 18.

B. Claim Group 2: Claims 11, 17, and 19

In addition to the reasons set forth above, which are incorporated herein, claims 11, 17, and 19 are directed towards treating chronic gastritis, as more specifically set forth therein. Hirai has not even been contended by the Examiner to teach or suggest a method of treating chronic gastritis, much less chronic gastritis caused by urease or *H.Pylori*. Accordingly, Hirai fails to anticipate claims 11, 17, and 19.

C. Claim Group 3: Claim 14

In addition to the reasons set forth above, which are incorporated herein, claim 14 is directed to the method of claim 9 for treating gastric mucosa injury caused by urease, the method further comprising administering at least one additional pharmacological active ingredient. Hirai has not been cited for teaching any such method comprising using multiple active ingredients. Accordingly, Hirai fails to anticipate claim 14.

VIII. Conclusion

For the reasons given above, pending claims 9-20 are allowable and reversal of the Examiner's rejection is respectfully requested.

To the extent any extension of time under 37 C.F.R. § 1.136 is required to obtain entry of this Appeal Brief, such extension is hereby respectfully requested. If there are any fees due under 37 C.F.R. §§ 1.16 or 1.17 which are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

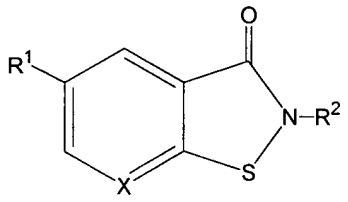
Dated: July 21, 2006

By: _____
Mark J. Feldstein
Reg. No. 46,693

IX. Claims Appendix to Appeal Brief Under Rule 41.37(c)(1)(viii)

Claims 1-8. (Cancelled)

9. A method of treating gastric mucosa injury caused by urease, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound represented by formula (1):



(1)

wherein R^1 represents a hydrogen atom or an amino group, R^2 represents a hydrogen atom, a lower alkyl group, or an acetyl group, and X represents a carbon atom or a nitrogen atom, or an adduct salt thereof.

10. A method according to claim 9, wherein the isothiazole compound is at least one selected from the group consisting of 1,2-benzoisothiazol-3(2H)-one, isothiazolo[5,4-b]pyridin-3(2H)-one, 5-amino-1,2-benzoisothiazol-3(2H)-one, N-methyl-1,2-benzoisothiazol-3(2H)-one and N-acetyl-1,2-benzoisothiazol-3(2H)-one.

11. A method according to claim 9, wherein the gastric mucosa injury comprises chronic gastritis.

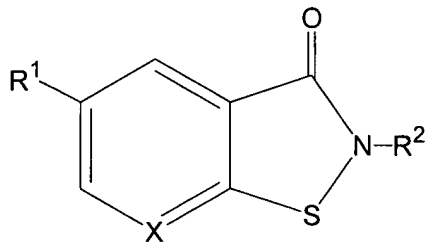
12. A method according to claim 9, wherein the gastric mucosa injury comprises gastroduodenal ulcer.

13. A method according to claim 9, comprising administering the isothiazole compound in a daily dose of from about 0.1 to 100 mg/kg.

14. A method according to claim 9, further comprising administering at least one additional pharmacologically active ingredient chosen from antibiotics, nitronidazole antiprotazoal agents, antiulcer drugs, and proton pump inhibitors.

15. A method according to claim 9, wherein the urease comprises urease produced by Helicobacter pylori.

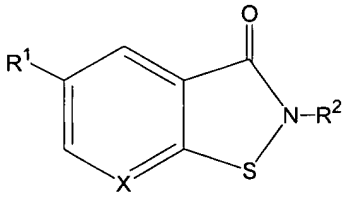
16. A method of treating gastric mucosa injury caused by Helicobacter pylori, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound represented by formula (1):



(1)

wherein R^1 represents a hydrogen atom or an amino group, R^2 represents a hydrogen atom, a lower alkyl group, or an acetyl group, and X represents a carbon atom or a nitrogen atom, or an adduct salt thereof.

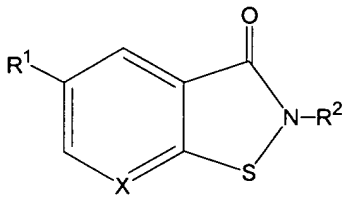
17. A method of chronic gastritis caused by urease, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound represented by formula (1):



(1)

wherein R^1 represents a hydrogen atom or an amino group, R^2 represents a hydrogen atom, a lower alkyl group, or an acetyl group, and X represents a carbon atom or a nitrogen atom, or an adduct salt thereof.

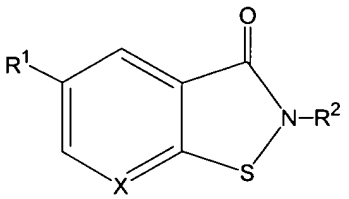
18. A method of treating a gastroduodenal ulcer caused by urease, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound represented by formula (1):



(1)

wherein R^1 represents a hydrogen atom or an amino group, R^2 represents a hydrogen atom, a lower alkyl group, or an acetyl group, and X represents a carbon atom or a nitrogen atom, or an adduct salt thereof.

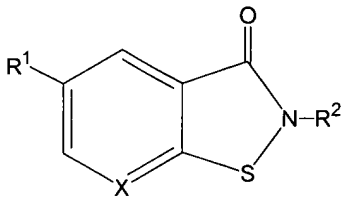
19. A method of chronic gastritis caused by Helicobacter pylori, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound represented by formula (1):



(1)

wherein R^1 represents a hydrogen atom or an amino group, R^2 represents a hydrogen atom, a lower alkyl group, or an acetyl group, and X represents a carbon atom or a nitrogen atom, or an adduct salt thereof.

20. A method of treating a gastroduodenal ulcer caused by Helicobacter pylori, which comprises administering to a person in need thereof a therapeutically effective amount of an isothiazole compound represented by formula (1):



(1)

wherein R^1 represents a hydrogen atom or an amino group, R^2 represents a hydrogen atom, a lower alkyl group, or an acetyl group, and X represents a carbon atom or a nitrogen atom, or an adduct salt thereof.

X. Evidence Appendix to Appeal Brief Under Rule 41.37(c)(1)(ix)

The references listed below are cited herein and included in this appendix. An indication of where each reference was made of record is provided in square brackets after each citation.

A. "Reduce Your Risk" brochure from the American Gastroenterological Association and American Pharmacists Associations. [Submitted by Applicant on Oct. 14, 2004, as an Attachment to the Amendment of that date, which was referenced and responded to by the Examiner's Dec. 23, 2004, Office Action.]

B. Huang, J.Q. et al., Lancent, 359:14-22 (2002). [Submitted by Applicant on June 20, 2005, with Amendment and RCE that were referenced and responded to by the Examiner's August 19, 2005, Office Action.]

C. Wolfe, M.M. et al.: N. Engl. J. Med., 340:1888-1899, 1888 (1999). [Submitted by Applicant on June 20, 2005, with Amendment and RCE that were referenced and responded to by the Examiner's August 19, 2005, Office Action.]

D. Wallace, J.L. et al.: Gastroenterology, 119:706-714, 713 (2000). [Submitted by Applicant on June 20, 2005, with Amendment and RCE that were referenced and responded to by the Examiner's August 19, 2005, Office Action.]

E. Langenbach, R. et al.: Cell, 83:483-492, 484 (1995). [Submitted by Applicant on June 20, 2005, with Amendment and RCE that were referenced and responded to by the Examiner's August 19, 2005, Office Action.]

F. "Prilosec®", in Physicians' Desk Reference ("PDR"), pages 633-638, at 637 (2004). [Submitted by Applicant on Oct. 14, 2004, as an Attachment to the Amendment of that date, which was referenced and responded to by the Examiner's Dec. 23, 2004, Office Action.]

and in most cases these problems can happen without warning. In fact, serious side effects of NSAIDs, such as stomach bleeding, result in nearly 103,000 hospitalizations and 16,500 deaths each year in the United States. That's more deaths than from AIDS and more than four times as many deaths as those from cervical cancer each year in the U.S.

Education is the first step in reducing your risk. REDUCE (Risk Education to Decrease Ulcer Complications and their Effects from NSAIDs) is a nationwide campaign created to help explain the potential harmful effects you could have if taking NSAIDs and help lower your risk for getting these problems.

About REDUCE

The REDUCE Campaign has been developed by the American Gastroenterological Association (AGA) and American Pharmacists Association (APhA) to increase awareness of the risks caused by over-the-counter and prescription NSAIDs. The goal of REDUCE is to reach physicians, pharmacists and consumers through public service announcements and other educational materials.

Originally launched by the AGA in 1998, REDUCE has been brought back due to the need for greater education about the risks of NSAIDs.

For additional information, log onto www.2REDUCE.org

This REDUCE Campaign is supported by an unrestricted educational grant from Pfizer Inc. AGA and APhA have complete editorial control of the content over the educational effort.

Produced by the AGA Press, a division of the AGA, dedicated to providing the latest, most accurate information in the field of Gastroenterology.

REDUCE CAMPAIGN

AGA Foundation

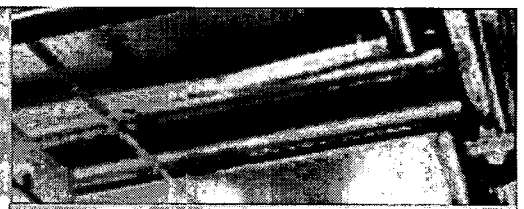
American Pharmacists Association

APhA

REDUCE YOUR RISK

Helping You Understand the Risks of Pain Relievers

REDUCE CAMPAIGN



REDUCE YOUR RISK

Every day more than 30 million people take over-the-counter and prescription drugs known as non-steroidal anti-inflammatory drugs or "NSAIDs" for effective relief from pain, headaches and arthritis.

These drugs have been around for a long time and have benefited many people. Although they are generally safe, NSAIDs, like all drugs, do have some potential side effects.

Many people don't know that NSAIDs can cause serious problems ranging from stomach upset to stomach bleeding, stomach pain, ulcers (a hole in the lining of the stomach) and even death. There is no medical test that can tell for sure if you will develop a problem,

What are NSAIDs?

NSAIDs are pain relievers that reduce pain and swelling at the site of injury. About twenty NSAIDs are available with a doctor's prescription. Three of these, ibuprofen, naproxen, and ketoprofen, also are available over-the-counter. The over-the-counter versions of some of these drugs are better known by names like Advil®, Motrin®, Aleve® and Orudis®. The only difference is that prescription versions are a higher strength than those purchased over-the-counter Other examples of NSAIDs include products with aspirin that are available over-the-counter, like Bayer® and Excedrin®. NSAIDs also can be found in common cold and flu medications such as Advil Cold and Sinus®, Dimetapp Sinus,® Motrin IB Sinus® and Aleve Cold and Sinus®. (Look for aspirin, naproxen or ibuprofen on the box or bottle to find out if your medicine contains an NSAID.)

Newer NSAIDs include meloxicam (Mobic®) and a class of drugs known as COX-2 specific inhibitors. Examples of these drugs include celecoxib (Celebrex®), valdecoxib (Bextra®) and rofecoxib (Vioxx®). These newer NSAIDs are available only through a doctor's prescription and may be safer for the stomach.

*Acetaminophen (Tylenol®) is not an NSAID.

How Do NSAIDs Cause Stomach Problems?

Problems caused by NSAIDs can range from mild stomach upset to stomach bleeding and ulcers. These problems occur because NSAIDs stop a substance in the body that protects the lining of your stomach from damage. Some people may be at higher risk for stomach problems.

Are You at Risk for NSAID-related Stomach Problems?

Everyone who takes NSAIDs can be at some risk for developing a stomach problem. Below are some factors that doctors and pharmacists think are "high-risk" and may make you most likely to have serious side effects.

To find out if you fall within this high-risk group, fill out this simple REDUCE Your Risk Checklist. Mark all that apply to you.

☐ Over the age of 60
☐ Have had previous ulcers
☐ Take steroid medications (such as prednisone)
☐ Take blood thinners (such as warfarin or Coumadin®)
☐ Consume alcohol on a regular basis
☐ Take NSAIDs in amounts higher than recommended on the bottle or by your doctor/pharmacist
☐ Take several different medications that contain NSAIDs
☐ Take NSAIDs for long periods of time

If you've checked any of the boxes in the checklist, take this brochure to your next doctor visit. Talk with your doctor about how to lower your risk. You can also review the checklist with your pharmacist for advice about how to minimize your risk. Remember that your pharmacist plays an important role in your healthcare team. Pharmacists help you understand your medications to make sure you are using them safely and effectively.

A 2003 survey showed that almost half of Americans who took over-the-counter NSAIDs in the last year took more than the recommended dose. This can happen by:

- taking the next dose sooner than directed on the label
- taking more tablets/capsules at a single time than recommended
- taking more than the recommended number of doses per day
- taking several different medications that contain NSAIDs at the same time

Even taking small amounts of over-the-counter NSAIDs can increase your risk of developing stomach problems. This includes taking daily, low-dose aspirin to prevent a heart attack, stroke, colon cancer or several other diseases.

The Warning Signs

In addition to knowing your risks, it's important to know the signs of a problem. See your doctor immediately if you experience:

- stomach pain
- dark black, tarry or bloody stools
- vomiting of blood or materials that look like coffee grounds

However, remember that about 80% of people who have a serious stomach problem as a result of taking a NSAID have no warning symptoms. Problems can even occur within one week of starting to take these pain relievers.

REDUCE Your Risk

There are steps you can take to reduce your risk for developing a serious stomach problem. The following are some important tips to guide you when taking any over-the-counter or prescription pain reliever.

- Know your personal risk factors (review the "REDUCE Your Risk" Checklist).
- Since problems can develop even if you do not have any of the common risk factors, talk to a doctor or pharmacist before you begin taking any medication. Also, ask questions and tell your doctor or pharmacist if you have any side effects.
- Read the label on your medications and follow the instructions. Know all the ingredients in your medications, how much to take (dose) and length of time (duration) you can safely take the product. Talk with your doctor before taking any pain reliever for more than 10 days.
- Take a medication only as directed and know its side effects. Look for side effects on the label or box of every medication bottle.
- Never use prescription and over-the-counter pain relievers at the same time unless directed by your doctor or pharmacist.
- Write down all medications and dietary supplements that you are taking. Be sure to include all vitamin, mineral and herbal supplements. Share the list with your doctor or pharmacist, they can help you avoid drug interactions or ingredient duplications.
- Avoid or limit use of alcohol when taking any pain medication.
- Talk to your doctor or pharmacist about medications that can reduce your risk for developing stomach problems when taking NSAIDs. Medications that decrease acid in your stomach, called proton pump inhibitors, can reduce your risk of stomach problems with NSAIDs. Examples of these medicines are lansoprazole (Prevacid®) and omeprazole (Prilosec®). Another medication, misoprostol (Cytotec®), is designed specifically to be taken with a NSAID to help reduce ulcers. Other options include taking acetaminophen (Tylenol®) instead of a NSAID or taking one of the newer NSAIDs, including meloxicam (Mobic®) or COX-2 inhibitors, which may cause fewer stomach problems. Examples of COX-2 inhibitors include celecoxib (Celebrex®), valdecoxib (Bextra®) and rofecoxib (Vioxx®). Also, consider not taking a NSAID at all.
- Talk with your doctor or pharmacist before beginning daily, low-dose aspirin.
- Talk with your doctor about pain that does not go away.
- Talk to your doctor or pharmacist if you are unsure if a drug contains a NSAID.

Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis

Jia-Qing Huang, Subbaramiah Sridhar, Richard H Hunt

Summary

Background The relation between *H pylori* infection and use of non-steroidal anti-inflammatory drugs (NSAIDs) in the pathogenesis of peptic-ulcer disease is controversial. We undertook a meta-analysis to address this issue.

Methods By computer and manually we sought observational studies on the prevalence of peptic-ulcer disease in adult NSAID takers or the prevalence of *H pylori* infection and NSAID use in patients with peptic-ulcer bleeding. Summary odds ratios were calculated from the raw data. Tests for homogeneity were done.

Findings Of 463 citations identified, 25 studies met inclusion criteria. In 16 studies of 1625 NSAID takers, uncomplicated peptic-ulcer disease was significantly more common in patients positive than in those negative for *H pylori* (341/817 [41·7%] *vs* 209/808 [25·9%]; odds ratio 2·12 [95% CI 1·68–2·67]). In five controlled studies, peptic-ulcer disease was significantly more common in NSAID takers (138/385 [35·8%]) than in controls (23/276 [8·3%]), irrespective of *H pylori* infection. Compared with *H pylori* negative individuals not taking NSAIDs, the risk of ulcer in *H pylori* infected NSAID takers was 61·1 (9·98–373). *H pylori* infection increased the risk of peptic-ulcer disease in NSAID takers 3·53-fold in addition to the risk associated with NSAID use (odds ratio 19·4). Similarly, in the presence of risk of peptic-ulcer disease associated with *H pylori* infection (18·1), use of NSAIDs increased the risk of peptic-ulcer disease 3·55-fold. *H pylori* infection and NSAID use increased the risk of ulcer bleeding 1·79-fold and 4·85-fold, respectively. However, the risk of ulcer bleeding increased to 6·13 when both factors were present.

Interpretation Both *H pylori* infection and NSAID use independently and significantly increase the risk of peptic ulcer and ulcer bleeding. There is synergism for the development of peptic ulcer and ulcer bleeding between *H pylori* infection and NSAID use. Peptic-ulcer disease is rare in *H pylori* negative non-NSAID takers.

Lancet 2002; **359:** 14–22
See Commentary page 3

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Introduction

The relation between infection with *Helicobacter pylori* and use of non-steroidal anti-inflammatory drugs (NSAIDs) in the pathogenesis of peptic-ulcer disease is controversial, because studies examining these two risk factors in this disorder have had conflicting results.[1–4] From conventional thinking, the presence of both these well-established risk factors for peptic-ulcer disease would be expected to increase the risk of the disease. However, this was not the case in several observational studies of patients taking NSAIDs, in which peptic-ulcer disease was less frequently diagnosed when *H pylori* infection was present than in patients without the infection.[5,6] Conflicting results have also been reported from randomised controlled clinical trials on whether eradication of *H pylori* infection retards ulcer healing[7,8] or reduces the risk of developing peptic-ulcer disease in NSAID takers.[9,10]

The discrepancies probably reflect a complex relation between *H pylori* infection and NSAID-associated gastropathy as well as methodological heterogeneity between studies. For example, study populations have differed in terms of NSAID exposure, the controls used for comparison, and the definition of ulcer size.[11–18] Therefore, there are four possible situations for *H pylori* infection and NSAID-associated gastropathy: no interaction, or additive, synergistic, or antagonistic effects between the two risk factors. The aims of this analysis were to review systematically the literature on the relation between *H pylori* infection and NSAID-associated gastropathy; to assess the presence and magnitude of any possible interaction on peptic-ulcer disease between these two risk factors; to examine any possible interaction between the two risk factors with respect to the site of ulcer or ulcer bleeding; and to explore any sources of heterogeneity between the published studies.

Methods

Design and procedures

A computerised literature search was done in the MEDLINE, PubMed, and Cochrane databases for relevant systematic reviews published in any language between 1984 and October 2000, with the following MeSH terms and/or textwords: meta-analysis, systematic review, overview, NSAIDs, and *pylori*. 15 potentially relevant citations were identified. By previously described criteria[19,20] and guidelines for the application of meta-analysis in epidemiological studies,[21] these reports were examined critically by one of the authors (J-QH). No review described a systematic search strategy, and methods to include reviewed articles and assessment of study validity and appropriate statistical analyses were not used. Thus, none of these reviews could be classified as a systematic review or meta-analysis. Our meta-analysis was therefore justified.

The following inclusion criteria were used: observational (cross-sectional, case-control, or cohort) studies investigating the prevalence of peptic-ulcer disease in adult patients taking NSAIDs or the

prevalence of *H pylori* infection and NSAID use in patients with peptic-ulcer bleeding, and the relation between *H pylori* infection and NSAID-associated peptic-ulcer disease; documentation of peptic-ulcer disease or ulcer bleeding was required by endoscopy; and *H pylori* infection had to be confirmed by histology, culture, serology, or urea breath test. Duplicate publications or studies published only in abstract form were excluded. We also excluded studies in which patients had reported recent use (3–4 weeks before study entry) of antibiotics or anti-ulcer drugs or a history of gastric surgery.

For the analysis of ulcer bleeding, in addition to these exclusion criteria, we applied an additional criterion to exclude studies that allowed enrolment of patients with non-ulcer gastrointestinal bleeding or gastric tumours and those receiving corticosteroids or anticoagulants.

The following search terms (both as MeSH terms and as keywords) were used to identify potentially relevant primary studies in the three databases mentioned above, without language restriction: NSAIDs, *pylori*, and ulcer or ulcer bleeding. A search on links to related articles was also done wherever available. A recursive hand-search of the references of all articles reviewed and of the retrieved original studies was done to look for studies not identified by the computer search. The following journals were manually searched for potentially relevant articles from January 1989 to October 2000: *Gastroenterology, Gut, American Journal of Gastroenterology, Alimentary Pharmacology and Therapeutics, Digestive Disease Sciences, European Journal of Gastroenterology and Hepatology, Scandinavian Journal of Gastroenterology, Journal of Clinical Gastroenterology, The Lancet, New England Journal of Medicine, Archives of Internal Medicine, British Medical Journal, JAMA, Annals of Internal Medicine,* and *Quarterly Journal of Medicine.*

The title and abstract of all potentially relevant studies were screened for their relevance to the study question before retrieval of full articles. However, full articles were also scrutinised for relevance if the title and abstract were ambiguous.

All searches were made independently by two reviewers (J-QH and SS).

Data were extracted from each study by J-QH and SS by means of a structured spreadsheet. In the case of disagreement, a third reviewer (RHH) was consulted. Major items were the primary question of an individual study, study design, characteristics of case and control populations, major exclusion criteria, definition of ulcer or ulcer bleeding, diagnostic method for ulcer or ulcer bleeding, site of ulcer, definition of NSAID use, type of NSAID, test for *H pylori* infection, total number of cases and controls, percentage of smokers, concurrent treatment, and prevalence of *H pylori* infection and ulcer.

The original investigators were contacted for further information on the site of ulcer or *H pylori* status where necessary.[11,18,22,23]

To assess the validity of each study, the following criteria were applied, modified from the guidelines for reading case-control studies proposed by Lichtenstein and colleagues:[24] an explicit statement of the research question and its relevance to the question of this meta-analysis; the methods for identification of cases and controls and their matching techniques; a clear statement of exclusion criteria for cases and controls; definition of NSAID exposure and peptic ulcer; the methods of data collection; and a description of analytical methods and sample size. To avoid subjective assessments, we did not

Ref	Design	Primary question	NSAID takers	Mean age (years)	Controls	Definition of NSAID use	Ulcer size
11	CC	Effect of *H pylori* on NSAID-related gastropathy	96 RA	63·1	96 dyspeptic patients matched by age and sex	Chronic use	Any size
13	CC	Prevalence of *H pylori* and GI mucosal lesions in NSAID takers	96 IHD	48	50 non-IHD patients matched by age	Daily aspirin >4 weeks	≥0·5 cm
12	Cohort	Interaction between *H pylori* and NSAIDs on GI mucosal damage	38 healthy volunteers	23·5	13 healthy individuals matched by age and sex	Daily use for 4 weeks	≥0·5 cm
18	CC	Mucosal blood flow in NSAID users and effect of *H pylori*	70 RA/OA	54*	17 dyspeptic patients, matched by age and sex†	>4 weeks	≥0·5 cm
14	CC	Relation of *H pylori* to gastric lesions in NSAID takers	85 RA	53	100 non-RA patients matched by age and sex	Chronic use >1 month	Not given
15	CC	Interaction between *H pylori* and NSAIDs on PUD	99 patients for endoscopy	57·5	331 patients, unmatched	Regular use before or within 1 month	≥0·5 cm
16	CC	Interaction between *H pylori* and NSAIDs on GI mucosal damage	76 RA	59	97 patients with abdominal symptoms, unmatched	Chronic use ≥3 months	Not given
17	CC	Interaction between *H pylori* and NSAIDs on gastric mucosa	174 RA	59*	44 RA, non NSAID users, unmatched	>4 weeks	≥0·5 cm
62	CS‡	Interaction between *H pylori* and NSAIDs on PUD	181 RD	61·5	Not available	Chronic use >3 months	≥0·5 cm
58	CS	Interaction between *H pylori* and NSAIDs on GI mucosal damage	128 dyspeptic patients	79·5	Not available	Any time in the 7 days before	Not given
7	CS	*H pylori* eradication on healing PUD in NSAID takers	246 RD	57·5	Not available	Daily use >4 weeks	≥0·5 cm
59	CS	Interaction between *H pylori* and NSAIDs on PUD	82 RD	54	Not available	Chronic use >3 months	≥0·5 cm
60	CS	Interaction between *H pylori* and NSAIDs on PUD	75 RA/OA	21–75§	Not available	Chronic use	≥0·5 cm
5	CS¶	Interaction between *H pylori* and NSAIDs on PUD	50 RA	65*	Not available	Daily use >6 months	≥0·5 cm
61	CS	Relation of *H pylori* to GI mucosal damage	85 RD	54	Not available	>8 months	Not given
63	CS	Relation of *H pylori* to GI mucosal damage	52 RA	52·8	Not available	Not given	Not given

CC=case control; RA=rheumatoid arthritis; GI=gastrointestinal; IHD=ischaemic heart disease; OA=osteoarthritis; PUD=peptic-ulcer disease; CS=cross-sectional; RD=rheumatoid diseases. *Median. †No data on *H pylori* status for controls. ‡Patients were from one group of a previously finished randomised trial. §Range. ¶Designed as case-control, but no endoscopy was done in controls.

Table 1: **Studies examining the relation between *H pylori* infection and NSAID use in patients with uncomplicated peptic-ulcer disease (listed in order of year of publication)**

Study ref	Odds ratio (95% CI)	Variance	Weight of study	Contribution to Q
14	0·74 (0·28–1·96)	0·25	3·98	13·16
18	4·27 (0·97–18·8)	0·57	1·75	0·006
12	21·9 (0·39–1216)	4·20	0·24	0·60
13	18·1 (6·17–53·0)	0·30	3·32	6·37
11	7·59 (3·05–18·9)	0·22	4·61	1·23
All	..	5·54	13·9	21·37

Cochrane Q 21·37, df=4, p<0·001.

Table 2: **Contribution of each study to the Cochrane Q, the statistical test of heterogeneity in a fixed-effects model**

generate an overall quality score, but validity criteria were used to rank studies. For example, a study with a clearly defined control group matched for age, sex, or both would be ranked more highly than one with a poorly matched control group or no controls. Disagreements were resolved by discussion and consensus between the researchers.

The following considerations were applied to determine the combinability of the individual studies for meta-analysis: study design, matching techniques in case-control studies, methods used for measuring outcome, and the biological plausibility. We also took into account the differences between individual results and the summary estimate of the odds ratio and the results of the tests for homogeneity.

Statistical analysis

The following statistical techniques were used to analyse the data, where appropriate. Summary odds ratio and 95% CI were calculated from the raw data of the selected studies by the method of DerSimonian and Laird in a random-effects model. The Breslow-Day method was used to test for homogeneity under the null hypothesis that the odds ratios were consistent across the selected studies. However, in the presence of statistical heterogeneity, we searched for the sources of any possible clinically important heterogeneity (ie, methodological or biological heterogeneity). We did not simply exclude outliers on the basis of the statistical test of homogeneity, because heterogeneity is expected rather than the exception in meta-analysis in epidemiological studies.[25,26]

Subgroup or sensitivity analyses under a random-effects model were carried out where appropriate.

The measurement of agreement between observers was expressed as the κ coefficient (PC agree). All other statistical analyses were done with EasyMa Software for Meta-analysis (EasyMa 2000).

Results

Publications

The literature search in the three databases generated 463 citations, and screening of citation titles and abstracts identified 61 potentially relevant studies for full article retrieval. Of these, 36 studies were subsequently excluded: *H pylori* eradication study;[8–10] treatment trial with omeprazole or H_2-receptor antagonists or misoprostol;[27–29] no baseline endoscopy data;[30] no raw data on peptic-ulcer disease or *H pylori* status;[31–50] inclusion of patients negative for *H pylori* only;[51] inclusion of patients recently exposed to H_2-receptor antagonists;[52,53] all patients had peptic-ulcer disease;[54,55] no contemporary control in patients with bleeding peptic-ulcer disease;[22] patients with ulcer perforation;[56] and review article.[57]

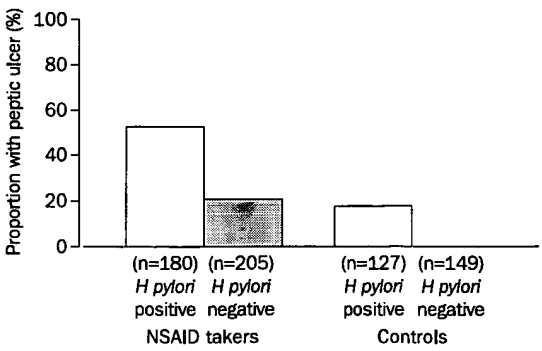

Figure 1: **Prevalence of peptic-ulcer disease among NSAID takers and controls by *H pylori* status**

Manual search of the references of the retrieved articles and major relevant medical journals did not yield any new studies.

The inter-observer agreement on study selection was high (κ=0·946).

Uncomplicated peptic-ulcer disease

16 studies provided raw data on the prevalence of peptic-ulcer disease in 1633 NSAID takers (table 1).[5,7,11–18,58–63] However, data on *H pylori* status were available for only 1625 patients. The pooled frequency of peptic-ulcer disease was 41·7% (341/817) in *H pylori* positive NSAID takers and 25·9% (209/808) in NSAID takers negative for *H pylori*, which gives a summary odds ratio of 2·12 (95% CI 1·68–2·67; Breslow-Day test p=0·43). Similar results were found when studies were grouped by study design, with a summary odds ratio of 3·53 (2·16–5·75) for controlled studies with matching for age, sex, or both, and 1·83 (1·41–2·38) for non-controlled studies.

Because individual patients' data were not available, analysis of age-adjusted prevalence of *H pylori* infection was not possible. However, plotting of study-specific odds ratios against mean or median age in each study did not identify any association between age and study findings (Pearson correlation coefficient –0·402, p=0·138).

Eight controlled studies compared the frequency of uncomplicated peptic-ulcer disease in NSAID takers and

Study ref	PUD in NSAID takers			PUD in non-NSAID takers		
	Number of cases/total		Odds ratio (95% CI)	Number of cases/total		Odds ratio (95% CI)
	H pylori positive	*H pylori* negative		*H pylori* positive	*H pylori* negative	
14	3/26	4/59	1·79 (0·37–8·66)	11/59	0/41	38·5 (0·72–2071)
18	17/30	10/40	3·92 (1·42–10·9)	2/13	0/4	3·40 (0·05–246)
12	9/24	2/14	3·60 (0·65–19·9)	0/8	0/5	0·64 (0·002–181)
13	49/65	11/31	5·57 (2·20–14·1)	4/13	0/37	68·5 (1·14–4124)
11	17/35	16/61	2·66 (1·11–6·37)	6/34	0/62	55·1 (0·99–3078)
Summary			3·53 (2·16–5·75)			18·1 (2·64–124)

PUD=peptic-ulcer disease. Breslow-Day test for homogeneity gave p=0·72 for PUD in NSAID takers, and p=0·32 for that in non-NSAID takers.

Table 3: **Analysis of studies with controls matched for age and sex by *H pylori* status**

Study ref	PUD in NSAID takers			PUD in non-NSAID takers		
	Cases/total		Odds ratio (95% CI)	Cases/total		Odds ratio (95% CI)
	H pylori positive	*H pylori* negative		*H pylori* positive	*H pylori* negative	
18	17/30	10/40	3·92 (1·42–10·9)	2/13	0/4	3·40 (0·05–246)
12	9/24	2/14	3·60 (0·65–19·9)	0/8	0/5	0·64 (0·002–181)
13	49/65	11/31	5·57 (2·20–14·1)	4/13	0/37	68·5 (1·14–4124)
11	17/35	16/61	2·66 (1·11–6·37)	6/34	0/62	55·1 (0·99–3078)

PUD=peptic ulcer disease. Breslow-Day test for homogeneity gave p=0·73 for PUD in NSAID takers and p=0·25 in non-NSAID takers. Odds ratio for *H pylori* positive NSAID takers *vs H pylori* negative NSAID takers 3·79 (2·27–6·34); for *H pylori* positive NSAID takers *vs H pylori* positive non-NSAID takers 5·62 (2·73–11·6); for *H pylori* positive NSAID takers *vs H pylori* negative non-NSAID takers 77·3 (10·3–581); for *H pylori* negative NSAID takers *vs H pylori* negative non-NSAID takers 21·6 (2·82–166); and for *H pylori* positive non-NSAID takers *vs H pylori* negative non-NSAID takers 14·4 (1·60–129).

Table 4: **Sensitivity analysis of controlled studies with matching for age, sex, or both, by *H pylori* status**

non-NSAID takers (table 1).[11–18] NSAID takers and controls were not matched by age in three of these studies.[15–17] Because *H pylori* infection is age dependent, we therefore analysed the prevalence of *H pylori* infection only in the remaining five studies.[11–14,18] In the study by Kordecki and colleagues,[13] two age-matched control groups were used for comparison with the NSAID takers. One consisted of patients with a similar primary diagnosis of disease to the NSAID takers, but with a history of peptic-ulcer disease. Furthermore, these controls might have received anti-ulcer treatment before study entry and cannot be considered as true controls. The other control group consisted of patients about to undergo surgery who had no history of peptic-ulcer disease. For our analysis, the latter control group was used.

Overall, *H pylori* infection was diagnosed in 46·8% (180/385) of the NSAID takers and 46·0% (127/276) of the controls. There was no significant difference in the pooled prevalence of the infection between the two groups (summary odds ratio 0·88 [95% CI 0·28–2·79], Breslow-Day test p<0·001). However, peptic-ulcer disease was significantly more common in NSAID takers than in controls (138/385 [35·8%] *vs* 23/276 [8·3%]; summary odds ratio 5·14 [1·35–19·6]; Breslow-Day test p<0·001) irrespective of *H pylori* infection.

Table 2 shows study-specific odds ratios of the five controlled studies[11–14,18] in a fixed-effects model, and the contribution of each study to the test of heterogeneity. The results show that the heterogeneity was caused predominantly by Caselli and colleagues' study,[14] because no significant heterogeneity was found after exclusion of that study (Breslow-Day test p=0·39).

In the study by Caselli and colleagues,[14] the exclusion criteria were not provided for the selection of controls. We could not be sure, therefore, whether patients with a history of peptic-ulcer disease were included in the control group. A sensitivity analysis that excluded this study gave a summary odds ratio of 9·41 (5·05–17·5).

Figure 1 illustrates the prevalence of peptic-ulcer disease in NSAID takers and controls by *H pylori* status.

The risk of peptic-ulcer disease associated with *H pylori* infection, without NSAID exposure, was calculated by comparing the difference in the frequency of peptic-ulcer disease between controls who were positive and negative for *H pylori* (odds ratio 18·1 [2·64–124]; table 3).[11–14,18]

The risk of peptic-ulcer disease associated with NSAID use, without *H pylori* infection, was estimated by comparing the difference in the prevalence of peptic-ulcer disease between *H pylori* negative NSAID takers and *H pylori* negative controls (odds ratio 19·4 [3·14–120]).[11–14,18]

In the presence of *H pylori* infection, the use of NSAIDs increased the risk of peptic-ulcer disease 3·55-fold (1·26–9·96). Similarly, in the presence of NSAID exposure, *H pylori* infection increased the risk of peptic-ulcer disease 3·53-fold (2·16–5·75; table 3).[11–14,18] However, when the comparison was between NSAID takers with *H pylori* infection and controls without the infection, the risk of peptic-ulcer disease increased to 61·1 (9·98–373).

Table 4 shows the results of sensitivity analysis with Caselli and colleagues' study excluded.

Among the five controlled studies with matching for age, sex, or both, four studies provided data on the site of the ulcer.[11,12,14,18] Table 5 gives the frequency of gastric and duodenal ulcer by *H pylori* status in the four studies and the estimated risk of developing a gastric or duodenal ulcer.

Bleeding peptic-ulcer disease

The literature search identified nine case-control studies assessing the prevalence of *H pylori* infection and NSAID use in 893 patients with bleeding peptic ulcer and 1002 controls without bleeding (table 6).[23,64–71]

Overall, the prevalence of *H pylori* infection was 73·6% (657/893) in the cases and 67·3% (674/1002) in the controls, yielding a summary odds ratio of 1·67 (95% CI 1·02–2·72; Breslow-Day, p<0·001; figure 2). Histology, rapid urease test, and culture have shown significantly higher false-negative rates than serology for diagnosis of *H pylori* infection in patients with bleeding peptic ulcers.[72,73] We therefore undertook subgroup

Study ref	Gastric ulcer				Duodenal ulcer			
	NSAID takers: cases/total		Non-NSAID takers: cases/total		NSAID takers: cases/total		Non-NSAID takers: cases/total	
	H pylori positive	*H pylori* negative	*H pylori* positive	*H pylori* negative	*H pylori* positive	*H pylori* negative	*H pylori* positive	*H pylori* negative
14	1/26 (4%)	4/59 (7%)	2/59 (3%)	0/41	2/26 (8%)	0/59	9/59 (15%)	0/41
11	13/35 (37%)	16/61 (26%)	3/34 (9%)	0/62	4/35 (11%)	3/61 (5%)	3/34 (9%)	0/62
12	3/24 (13%)	2/14 (14%)	0/8	0/5	6/24 (25%)	0/14	0/8	0/5
18	10/30 (33%)	5/40 (13%)	0/13	0/4	7/30 (23%)	5/40 (13%)	2/13 (15%)	0/4
Odds ratio (95% CI)	1·72 (0·92–3·20)		4·07 (0·39–42·9)		2·77 (1·12–6·88)		9·14 (1·02–81·8)	

Breslow-Day test for homogeneity gave p=0·4 for gastric ulcer in NSAID takers; p=0·41 for gastric ulcer in non-NSAID-takers; p=0·42 for duodenal ulcer in NSAID takers; and p=0·51 for duodenal ulcer in non-NSAID-takers.

Table 5: **Analysis of effects of *H pylori* infection and NSAID use on the site of peptic ulcer**

Study ref	Primary question	Study design	Cases and diagnosis of GI bleeding	Mean age (years)	Controls	NSAID use
64	Interaction between *H pylori* and NSAIDs in PU bleeding	CC	185 patients with bleeding PU verified by clinical and endoscopic findings	55	185 hospital controls, matched by age and sex	<1 week before entry
23	Role of *H pylori* and NSAIDs in PU bleeding	CC	97 patients with haematemesis or melaena 1 week before entry and with endoscopic stigma of recent bleeding	66	97 patients undergoing endoscopy without PU, matched by age and sex	<7 days of endoscopy
65	Role of *H pylori* in NSAID associated GI bleeding	CC	73 patients with haematemesis, melaena or anaemia with a loss of >3 g/dL haemoglobin and with endoscopic stigma of recent bleeding	80	73 non-bleeding patients, matched by endoscopic diagnosis, age and sex	<7 days of endoscopy
66	Interaction between *H pylori* and NSAIDs in upper GI bleeding	CC	72 patients with endoscopically verified PU bleeding	69	72 non-GI patients, matched by age, sex, and race	<1 week before entry
67	Role of *H pylori* in PU bleeding in NSAID users	CC	132 NSAID users with endoscopically verified GI bleeding from PU or haemorrhagic gastritis	72*	136 NSAID users with no sign of GI bleeding, matched by age and sex	<1 week before entry
68	Interaction between *H pylori* and NSAIDs in PU bleeding	CC	82 patients with PU bleeding verified by endoscopy	75	93 hospital controls, matched by age and sex	Not given
69	Role of *H pylori* and NSAID use in GU bleeding	CC	100 patients with GU bleeding verified by clinical and endoscopic findings	67	117 patients with non-bleeding GU, matched by sex	Within 4 weeks before entry
70	Prevalence of *H pylori* and relation to NSAIDs in PU bleeding	CC	106 patients with haematemesis or melaena from PU verified by endoscopy	68	30 healthy volunteers, matched by region and socioeconomic status	<1 week before entry
71	Prevalence of *H pylori* and NSAID use in PU bleeding	CC	46 patients with endoscopically diagnosed PU bleeding	23–83†	199 patients with non-bleeding PU, matching data unknown	1 month before entry

GI=gastrointestinal; PU=peptic ulcer; CC=case-control; GU=gastric ulcer; *Median. †Range.

Table 6: **Studies examining the relation between *H pylori* infection and NSAID use in patients with peptic-ulcer bleeding**

analyses according to *H pylori* testing methods. The summary odds ratio was estimated at 2·16 (1·54–3·04) for studies that used serology (figure 3).[66–68,70] The test of homogeneity was not significant for these studies (Breslow-Day p=0·32). The summary odds ratio for studies that used non-serological tests was 1·24 (0·50–3·08), with a highly significant test of homogeneity (Breslow-Day p<0·001).[23,64,65,69,71]

Seven of the nine studies provided comparable data on the prevalence of NSAID use in cases and controls.[23,64–66,68,69,71] The prevalence of NSAID use was 59·7% (391/655) in the cases and 27·4% (230/839) in the controls, giving a summary odds ratio of 4·79 (3·78–6·06; Breslow-Day p=0·3).

Of the nine studies, six had controls matched for age, sex, or both.[23,64–68] The pooled prevalence of *H pylori* infection in these studies was 70·2% (450/641) in the cases and 56·1% (368/656) in the controls, yielding a summary odds ratio of 1·79 (0·97–3·32; Breslow-Day p<0·001), irrespective of the method of testing for *H pylori* infection. In subgroup analyses on *H pylori* testing methods, the summary odds ratio for studies that used serology was 2·13 (1·38–3·31; Breslow-Day p=0·19).[66–68] For studies that used non-serological tests, the summary odds ratio was 1·42 (0·38–5·28; Breslow-Day p<0·001).[23,64,65]

Of the six case-control studies,[23,64–68] the pooled prevalence of NSAID use was 58·6% (357/609) in the cases and 23·5% (150/637) in the controls, giving a summary odds ratio of 4·85 (3·77–6·23; Breslow-Day p=0·21).

In the comparison between *H pylori* infected NSAID takers (64·5%, 149/231) and *H pylori* negative controls not taking NSAID (23·0%, 40/174), the risk of developing ulcer bleeding was 6·13 (3·93–9·56).[23,64] This value is almost the sum of the two odds ratios estimated for *H pylori* infection (1·79) and NSAID use (4·85).

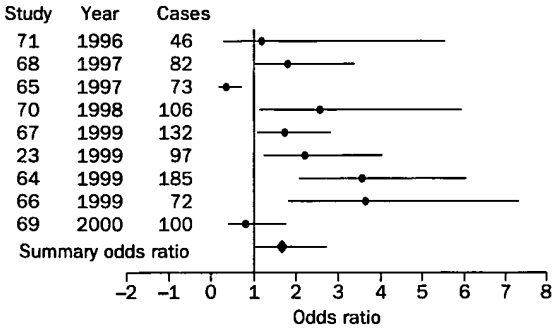

Figure 2: **Study-specific and summary odds ratios in all case-control studies assessing the prevalence of *H pylori* infection in patients with bleeding ulcers**

Odds ratio is represented by oval or diamond symbol; 95% CI by horizontal lines.

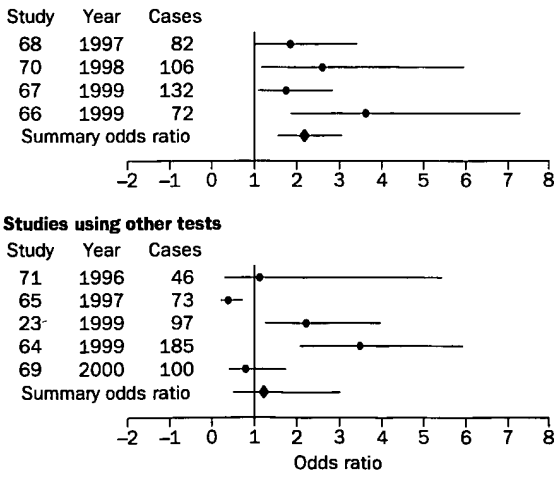

Figure 3: **Study-specific and summary odds ratios in studies using serology or other tests for *H pylori* infection**

Discussion

Previous studies have shown that *H pylori* infection or use of NSAIDs confers a three-fold to four-fold increased risk of peptic-ulcer disease.[74–76] However, whether the magnitude of the risk reported in these studies was for the individual or combined contribution of *H pylori* infection and NSAIDs to the development of peptic-ulcer disease or ulcer complications was not known.

We have found, in this systematic review, that a third of patients taking NSAIDs long term had gastric or duodenal ulcers, irrespective of *H pylori* status and study design. However, peptic-ulcer disease was significantly more common in *H pylori* infected NSAID takers than in takers without the infection, suggesting a possible interaction between NSAID use and *H pylori* infection for the development of peptic ulcer.

Because the prevalence of *H pylori* infection is significantly age dependent, assessment of the effect of *H pylori* infection on NSAID-associated peptic-ulcer disease in age-matched controlled studies is meaningful. In studies for which an age-matched control group was available, NSAID use increased the risk of peptic ulcer five-fold compared with non-NSAID takers, irrespective of *H pylori* status. However, *H pylori* infection increased the risk of peptic ulcer disease 3·5-fold and 18-fold in NSAID takers and controls, respectively. The odds ratio of 3·5 among NSAID takers is explained by the increased risk of peptic-ulcer disease in the presence of *H pylori* infection in addition to the risk associated with NSAID use (odds ratio 19·4). The extremely large odds ratios seen in the comparisons between control populations might have resulted from a zero event rate in *H pylori* negative controls, because statistical modelling by adding a constant pseudo-count of 1 to the analysis yielded a more meaningful and reliable odds ratio (6·36 [95% CI 2·21–18·3]). Nevertheless, the results are consistent with clinical and epidemiological data that peptic-ulcer disease is related primarily to *H pylori* infection and NSAID use.[77]

No peptic-ulcer disease was seen in patients without *H pylori* infection who were not taking NSAIDs in the five controlled studies (figure 1).[11–14,18] Therefore, this population is the true control population for assessment of any possible interaction between *H pylori* infection and NSAID use for the development of peptic-ulcer disease. As shown in this analysis, the effect of *H pylori* infection and NSAID use on peptic-ulcer disease, as shown by the magnitude of risk, was additive when *H pylori* infected NSAID takers were compared with the true controls; the results were confirmed by sensitivity analyses, which suggests a synergism for the development of peptic-ulcer disease between these two risk factors.

Another uncertainty is whether, in NSAID takers, *H pylori* infection is an important risk factor for gastric ulcer as it is for duodenal ulcer, on the basis of the existing evidence.[5,7,11–18,58–63] Most published studies did not separate patients by the location of ulcer and reported these ulcers together.[5,7,13,15–17,58–63] Our pooled analysis of four studies showed that *H pylori* infection is less closely associated with gastric ulcer than with duodenal ulcer in both NSAID takers and control groups,[11,12,14,18] although this result might have been caused by a small sample size. Nevertheless, the results suggest that NSAID use has a major role in the development of gastric ulcer, whereas duodenal ulcer is more closely related to *H pylori* infection. The mixed patient population may have contributed to the conflicting published results. Therefore, future studies should separate clearly patients with gastric ulcer from those with duodenal ulcer when examining the relation between *H pylori* infection and NSAID-associated peptic-ulcer disease.

There has been debate over the clinical importance of endoscopically detected gastric and duodenal ulcers associated with NSAID use because of a weak relation between endoscopically observed gastroduodenal mucosal lesions and symptoms.[78,79] However, previous studies have not taken *H pylori* infection into account.[76] Therefore, whether there is any relation between endoscopically observed ulcers and symptoms in patients infected with *H pylori* is not known. Thus, results from studies of patients with ulcer complications may provide more important and clinically relevant information on the interaction between *H pylori* infection and NSAID use. In this systematic review, we found that NSAID use (odds ratio 4·85) was significantly more common in patients with bleeding peptic ulcer than in controls, whereas *H pylori* infection (1·79) only marginally increased the risk of ulcer bleeding. However, when both risk factors coexist, the magnitude of the risk was additive (6·13), which suggests that both *H pylori* infection and NSAID use contribute to peptic-ulcer bleeding with NSAIDs having a major role, on the basis of the magnitude of the risk ratio.

The recent debate over the role of *H pylori* infection in NSAID-associated peptic-ulcer disease has been fuelled largely by the conflicting results from two randomised controlled clinical trials.[9,10] The differences between these two studies have been extensively reviewed elsewhere.[80,81] The panel summarises major differences in the methodology and findings. These differences are fundamental and may help to explain the contrasting conclusions from these two studies.

Whether eradication of *H pylori* infection retards ulcer healing in NSAID takers is also controversial.[7,8] Chan and colleagues reported, in a randomised clinical trial of 195 *H pylori* infected patients with NSAID-associated bleeding ulcer, that eradication of the infection did not impair ulcer healing compared with antisecretory treatment alone.[8] By contrast, Bianchi Porro and colleagues found that ulcer healing rate was reduced by *H pylori* eradication, although successful eradication of

Major differences between studies by Chan and colleagues[9] and Hawkey and colleagues[10]

Features	Chan et al[9]	Hawkey et al[10]
Population	Chinese	European
Long-term NSAID use	Excluded	Included
Ulcer history	Excluded	Included (6% more ulcer at entry in the eradication group)
Definition of ulcer	≥5 mm	≥3 mm
NSAID used	Naproxen	Various
Eradication regimen	1 week bismuth triple	1 week omeprazole triple
Follow-up period	2 months	6 months
Ulcer by randomisation		
Eradication	7%	44%
Controls	26%	47%
Ulcer by final *H pylori* status		
H pylori eradicated	2·5%	Not provided
H pylori persisted	26%	Not provided

the infection decreased ulcer recurrence by 15% during 6 months of follow-up compared with patients with persistent infection.[7] More recently, Chan and colleagues reported that *H pylori* eradication was as effective as omeprazole maintenance treatment for preventing ulcer rebleeding in users of low-dose aspirin, but not in patients taking naproxen,[82] which suggests that *H pylori* eradication cannot replace maintenance treatment with antisecretory agents in regular NSAID takers at high risk of ulcer bleeding.

The results of secondary analyses of two large cohort studies, ASTRONAUT and OMNIUM, are also difficult to interpret and compare with the findings of our analysis because of study design and the primary question of continuous maintenance treatment with antisecretory agents or misoprostol in long-term NSAID takers.[27,29] *H pylori* infection is known to increase the antisecretory effect of omeprazole,[83,84] which might partly explain the difference in ulcer remission between *H pylori* positive and negative patients.[80,81]

In this study, we identified several sources of heterogeneity through sensitivity and subgroup analyses, which might help explain the conflicting results and opinions previously published. For example, in the comparison of uncomplicated peptic-ulcer disease, unclear selection criteria for the control population used in Caselli and colleagues' study[14] accounted for more than half of the heterogeneity among the five controlled studies. In studies examining the relation between *H pylori* infection and NSAID use in ulcer bleeding, different testing methods for *H pylori* infection led to the heterogeneity in the overall and subgroup analyses.

There are likely to be other sources of heterogeneity that have not been identified in this analysis. There are also several limitations. For instance, the prevalence of *H pylori* infection could not be adjusted by age because of the lack of individual patients' data; NSAID takers had various underlying disorders, and different control populations were used. Furthermore, patients could have been exposed to different NSAIDs or aspirin with varying ulcerogenic potency. Different definitions of ulcer could also be a source of bias. Finally, the conclusions drawn from subgroup analyses might be limited by small sample sizes. Nevertheless, we believe that meta-analysis is a useful tool for systematically assessing the totality of evidence and to provide directions for future studies. The results of this analysis may provide grounds for a biologically meaningful argument that the interaction between *H pylori* infection and NSAID use in peptic-ulcer disease is consistent across different populations of patients and study designs.

In conclusion, *H pylori* infection and NSAID use independently increase the risk of peptic-ulcer disease and ulcer bleeding. There is synergism for the formation of peptic ulcer and ulcer bleeding between these two risk factors. Peptic-ulcer disease is rare in *H pylori* negative non-NSAID takers.

Contributors

Jia-Qing Huang and Richard Hunt initiated the project, contacted the original investigators for raw data, and wrote the article; Jia-Qing Huang and Subbaramiah Sridhar did the literature search, data extraction, and validity assessment. Jia-Qing Huang did all the statistical analyses; Richard Hunt was consulted on all issues and discrepancies during the process of the study.

Conflict of interest statement

None declared.

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Uses of error

Learning experiences

Chhanda Bewtra

The year was 1979. I had just finished my residency training and joined my department as a brand new junior pathologist full of enthusiasm and brimming with self-confidence. I was taught by the old masters, who proclaimed diagnostic pathology as the last word, with no room for doubt or error. Reality was quite different.

A 79-year-old heavy smoker presented with haemoptysis, chest pain, and a non-resolving large central lung mass. A bronchoscopy yielded large, atypical cells which I diagnosed as non-small-cell carcinoma and next day the entire lobe with the mass was resected. As I opened it up, I was aghast to see a large, organising infarct. The bronchial tree was clean. I couldn't see any tumours anywhere in the specimen. I submitted over 100 sections to be sure I was not missing something, but it remained stubbornly a benign infarct. I remember the first shock and heart-stopping fear when I realised that I had made a major mistake. Then the desperation, a meticulous search all over the specimen for a non-existent tumour, followed by intense fear that my budding career was doomed. Then shame, and finally a lack of self-confidence that dogged me for weeks. I showed all the slides to my colleagues. Everyone agreed, it was an error, a false-positive diagnosis of cancer. I had notified the clinician immediately. He was an experienced pulmonologist, and was not very perturbed. A large, non-resolving infarct like this needed to be resected. "No harm done", he said patronisingly, but I was so mortified that for a week I couldn't even visit the doctors' lounge, fearing everyone was talking about my shameful error. Obviously, as a neophyte, I was very self-conscious. I remember having an intense desire to go to the patient's room and apologise for my error. I decided this was not a good idea in such a litigious society. I still wish there were a safe outlet for physicians to acknowledge their errors.

The patient recovered uneventfully. I did a literature search and found only one report of a pulmonary infarct with atypical cytology. I wrote up a modest research proposal to study this phenomemon, first in a canine model and later in a prospective human study. Fortunately, this study earned me my first research grant and numerous publications describing this dangerous pitfall in the diagnosis of lung cancer. I learnt a lesson here, and by subsequent research and publications I have tried to educate my peers about my mistake. I still have the slides and use them to teach medical students and residents about errors and how to handle them. Looking back, I smile ruefully at my intense reaction.

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Review Article

Medical Progress

GASTROINTESTINAL TOXICITY OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

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ONE hundred years have passed since Felix Hoffman, working at Bayer Industries, reported the successful synthesis of acetylsalicylic acid as the first nonsteroidal antiinflammatory drug (NSAID).[1,2] At the suggestion of Hermann Dreser, Bayer's chief pharmacologist at the time,[3] the compound was called "aspirin" and was purported to represent a convenient mechanism for the delivery of salicylic acid in the treatment of rheumatic diseases, menstrual pain, and fever.[2] Approximately 40 years elapsed before Douthwaite and Lintott[4] provided endoscopic evidence that aspirin could cause gastric mucosal damage. Numerous reports have corroborated this observation,[5-8] and the introduction of more potent agents with an even greater propensity for toxic effects has increased the awareness of NSAID-induced gastroduodenal ulcer and provided impetus for the development of effective NSAIDs with a more favorable safety profile.

Starting in the early 1970s, numerous new NSAIDs were developed that were initially believed to be devoid of gastrointestinal toxicity, but few, if any, are entirely harmless. These agents constitute one of the most widely used classes of drugs, with more than 70 million prescriptions and more than 30 billion over-the-counter tablets sold annually in the United States.[9] Although NSAIDs are generally well tolerated, adverse gastrointestinal events occur in a small but important percentage of patients, resulting in substantial morbidity and mortality.

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EPIDEMIOLOGY OF GASTROINTESTINAL COMPLICATIONS

Because of the broad and nonspecific definitions of gastrointestinal disorders caused by the use of NSAIDs, as well as differences in patient populations, drugs, dosages, and periods of use, estimates of the prevalence of adverse effects vary greatly. In general, at least 10 to 20 percent of patients have dyspepsia while taking an NSAID, although the prevalence may range from 5 to 50 percent.[10,11] Within a six-month period of treatment, 5 to 15 percent of patients with rheumatoid arthritis can be expected to discontinue NSAID therapy because of dyspepsia.[11]

According to prospective data from the Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS), 13 of every 1000 patients with rheumatoid arthritis who take NSAIDs for one year have a serious gastrointestinal complication. The risk in patients with osteoarthritis is somewhat lower (7.3 per 1000 patients per year).[12]

The rate of NSAID-related serious gastrointestinal complications requiring hospitalization has decreased in recent years. The decrease may be due, at least in part, to extensive medical-education campaigns that have persuaded physicians to use newer, less toxic NSAIDs and non-NSAID analgesics in populations at high risk.[12]

The mortality rate among patients who are hospitalized for NSAID-induced upper gastrointestinal bleeding is about 5 to 10 percent.[13] An analysis of data from ARAMIS has shown that the mortality rate attributed to NSAID-related gastrointestinal toxic effects is 0.22 percent per year, with an annual relative risk of 4.21 as compared with the risk for persons not using NSAIDs.[12] Although the annual mortality rate is low, it must be emphasized that because a large number of patients are exposed to NSAIDs, often for extended periods of time, the risk over a lifetime is substantial. In the United States, for instance, it is estimated that NSAIDs are used regularly by at least 13 million people with various arthritides. On the basis of these conservative figures and ARAMIS data, the annual number of hospitalizations in the United States for serious gastrointestinal complications is estimated to be at least 103,000. At an estimated cost of $15,000 to $20,000 per hospitalization, the annual direct costs of such complications exceed $2 billion.[14]

It has been estimated conservatively that 16,500 NSAID-related deaths occur among patients with rheumatoid arthritis or osteoarthritis every year in the United States. This figure is similar to the number of deaths from the acquired immunodeficiency

syndrome and considerably greater than the number of deaths from multiple myeloma, asthma, cervical cancer, or Hodgkin's disease (Fig. 1).[12,15] If deaths from gastrointestinal toxic effects of NSAIDs were tabulated separately in the National Vital Statistics reports, these effects would constitute the 15th most common cause of death in the United States. Yet these toxic effects remain largely a "silent epidemic," with many physicians and most patients unaware of the magnitude of the problem.[12] Furthermore, the mortality statistics do not include deaths ascribed to the use of over-the-counter NSAIDs.

In a recent survey of 4799 Americans, 807 were identified who had taken NSAIDs (prescribed or over-the-counter drugs) at least twice in the past year for five or more consecutive days.[12] Approximately 45 percent of the group took NSAIDs for five or more consecutive days at least once per month, and 40 percent took both over-the-counter and prescribed NSAIDs. Nearly 75 percent of those who used NSAIDs regularly were either unaware of or unconcerned about possible gastrointestinal complications. In addition, almost two thirds of the regular users indicated that they would expect warning signs before the development of serious NSAID-induced complications. Only a minority of patients who have serious gastrointestinal complications report any antecedent dyspepsia.[11,13] In a study of patients with serious gastrointestinal complications, Singh et al.[11] found that although the proportion of patients with prior symptoms was only slightly higher than the proportion with no prior symptoms (2.7 percent vs. 2.0 percent), 81 percent of the patients reported no antecedent dyspepsia.

RISK FACTORS FOR GASTROINTESTINAL COMPLICATIONS

Because dyspeptic symptoms are not a reliable warning sign, it is important to identify factors that increase the risk of serious gastrointestinal complications and to determine methods for reducing this risk. A number of studies have been designed to identify patients who are most likely to have adverse effects of NSAID therapy (Table 1).

Advanced age has been consistently found to be a primary risk factor for adverse gastrointestinal events. The risk increases linearly with age.[15-20] Although previous reports suggested that the risk diminishes over time, a recent study indicates that the risk of NSAID-associated gastrointestinal hemorrhage remains constant over an extended period of observation.[12] Other risk factors that have been identified in multiple studies are higher doses of NSAIDs (including the use of two or more NSAIDs), a history of gastroduodenal ulcer or gastrointestinal bleeding, concomitant use of corticosteroids, serious coexisting conditions, and concomitant use of anticoagulants.[20-27] However, many of these studies are based on univariate analysis and do not consider the interactions among multiple factors and coexisting conditions.

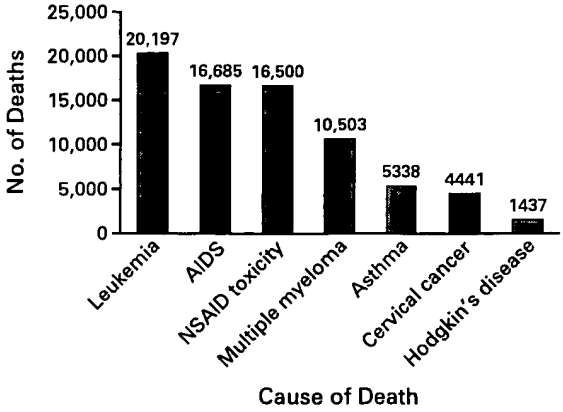

Figure 1. U.S. Mortality Data for Seven Selected Disorders in 1997. A total of 16,500 patients with rheumatoid arthritis or osteoarthritis died from the gastrointestinal toxic effects of NSAIDs. Data are from the National Center for Health Statistics and the Arthritis, Rheumatism, and Aging Medical Information System.[12]

TABLE 1. RISK FACTORS FOR THE DEVELOPMENT OF NSAID-ASSOCIATED GASTRODUODENAL ULCERS.*

Established risk factors
Advanced age (linear increase in risk)
History of ulcer
Concomitant use of corticosteroids
Higher doses of NSAIDs, including the use of more than one NSAID
Concomitant administration of anticoagulants
Serious systemic disorder
Possible risk factors
Concomitant infection with *Helicobacter pylori*
Cigarette smoking
Consumption of alcohol

*Information on risk factors is from Singh and Triadafilopoulus,[12] Bjorkman,[16] Longstreth,[17] Greene and Winickoff,[18] Gabriel et al.,[19] Griffin et al.,[20] Langman et al.,[21] Garcia Rodriguez and Jick,[22] Hallas et al.,[23] Silverstein et al.,[24] Hochain et al.,[25] Piper et al.,[26] Shorr et al.,[27] and Barkin.[28]

The identification of *Helicobacter pylori* infection as a factor in the development of peptic ulcer has raised the question of a possible synergistic relation between the presence of *H. pylori* infection and NSAID use. Although several studies[29-32] have found these two factors to be independent, two prospective studies have suggested a synergistic relation. Bianchi Porro et al.[33] used the combination of amoxicillin and omeprazole to treat NSAID users infected

with *H. pylori*. They found that the eradication of *H. pylori* did not affect the rate of ulcer healing. However, six months after the end of combination therapy, the cumulative rate of recurrent ulcers was 31 percent among the patients in whom *H. pylori* had been eradicated and 46 percent among those who were still infected. This difference was not statistically significant.

Chan et al.[34] found that the use of a regimen that included bismuth subcitrate to eradicate *H. pylori* significantly decreased the rate of ulcer development associated with the use of naproxen. In this study, gastroduodenal ulcers developed in 26 percent of *H. pylori*–infected persons, but in only 7 percent of those in whom the organism had been eradicated. The inclusion of bismuth in the drug regimen, however, makes the findings somewhat ambiguous, because bismuth can accumulate in the gastric mucosa and stimulate prostaglandin synthesis.[28] Most recently, Hawkey et al.[35] randomly assigned 285 patients with current ulcers or a history of ulcers who were using NSAIDs to combined treatment with omeprazole, clarithromycin, and amoxicillin or to treatment with omeprazole alone. They found that the eradication of *H. pylori* did not affect the rate of recurrent ulcer; in addition, ulcer healing was impaired even in the patients who were successfully treated with antibiotics for *H. pylori* infection. It thus appears that infection with *H. pylori* increases the risk of gastroduodenal mucosal injury associated with NSAID use only minimally, if at all.[28]

Singh et al.[36] recently proposed a simple, point-based algorithm that patients and their physicians can use to estimate the risk of an NSAID-related gastrointestinal complication. If confirmed by other investigators, this tool may help guide decisions about prescriptions for specific NSAIDs, the use of prophylactic agents, and the need for and frequency of patient monitoring.[36]

PATHOGENESIS OF NSAID-INDUCED GASTRODUODENAL MUCOSAL INJURY

Gastroduodenal mucosal injury develops when the deleterious effect of gastric acid overwhelms the normal defensive properties of the mucosa. Concepts about NSAID-induced gastroduodenal mucosal injury have evolved from a simple notion of topical injury to theories involving multiple mechanisms with both local and systemic effects (Fig. 2). The systemic effects are largely the result of the inhibition of endogenous prostaglandin synthesis.[37] Prostaglandin inhibition, in turn, leads to decreases in epithelial mucus, secretion of bicarbonate, mucosal blood flow, epithelial proliferation, and mucosal resistance to injury.[38,39] The impairment in mucosal resistance permits injury by endogenous factors, including acid, pepsin, and bile salts, as well as by exogenous factors such as NSAIDs and possibly ethanol and other noxious agents.

Topical Injury

Mucosal injury is initiated topically by the acidic properties of aspirin and many other NSAIDs. Because of a low dissociation constant, which varies according to the particular agent, these weak acids remain in their nonionized lipophilic form in the highly acidic gastric lumen. Such conditions favor migration through the gastric mucus across plasma membranes and into surface epithelial cells, where NSAIDs are dissociated into the ionized form, resulting in trapping of hydrogen ions.[37] NSAIDs can also cause topical mucosal damage by diminishing the hydrophobicity of gastric mucus, thereby allowing endogenous gastric acid and pepsin to injure the surface epithelium.[39] In addition, topical mucosal injury may occur as a result of indirect mechanisms, mediated through the biliary excretion and subsequent duodenogastric reflux of active NSAID metabolites.[40,41] For example, although sulindac is administered as a nontoxic prodrug, its active metabolite, sulindac sulfide, is excreted into the bile. On entry into the duodenum, sulindac sulfide causes topical injury to the mucosa by virtue of its acidic properties.

The Role of Prostaglandins

Topical injury caused by NSAIDs contributes to the development of gastroduodenal mucosal injury. However, the systemic effects of these agents appear to have the predominant role,[37,42,43] largely through the decreased synthesis of mucosal prostaglandins.[44] The use of enteric-coated aspirin preparations[44] and parenteral[45] or rectal[46] administration of NSAIDs in order to prevent topical mucosal injury has also failed to prevent the development of ulcers. Moreover, doses of aspirin as low as 30 mg are sufficient to suppress prostaglandin synthesis in the gastric mucosa.[47]

Prostaglandins are derived from arachidonic acid, which originates from cell-membrane phospholipids through the action of phospholipase A_2 (Fig. 3). The metabolism of arachidonic acid to prostaglandins and leukotrienes is catalyzed by the cyclooxygenase pathway and the 5-lipoxygenase pathway, respectively.[1,37] Two related but unique isoforms of cyclooxygenase, designated cyclooxygenase-1 and cyclooxygenase-2, have been demonstrated in mammalian cells.[48,49] Despite their structural similarities, they are encoded by distinct genes and differ with regard to their distribution and expression in tissues.[50,51] The cyclooxygenase-1 gene contains a promoter region without a TATA sequence and is primarily expressed constitutively. In contrast, the cyclooxygenase-2 gene is thought to be the inducible form that is nearly undetectable in most (but not all) tissues under normal physiologic conditions.

Cyclooxygenase-1 appears to function as a "housekeeping" enzyme in most tissues, including the gastric mucosa, the kidneys, and the platelets, whereas the expression of cyclooxygenase-2 can be induced

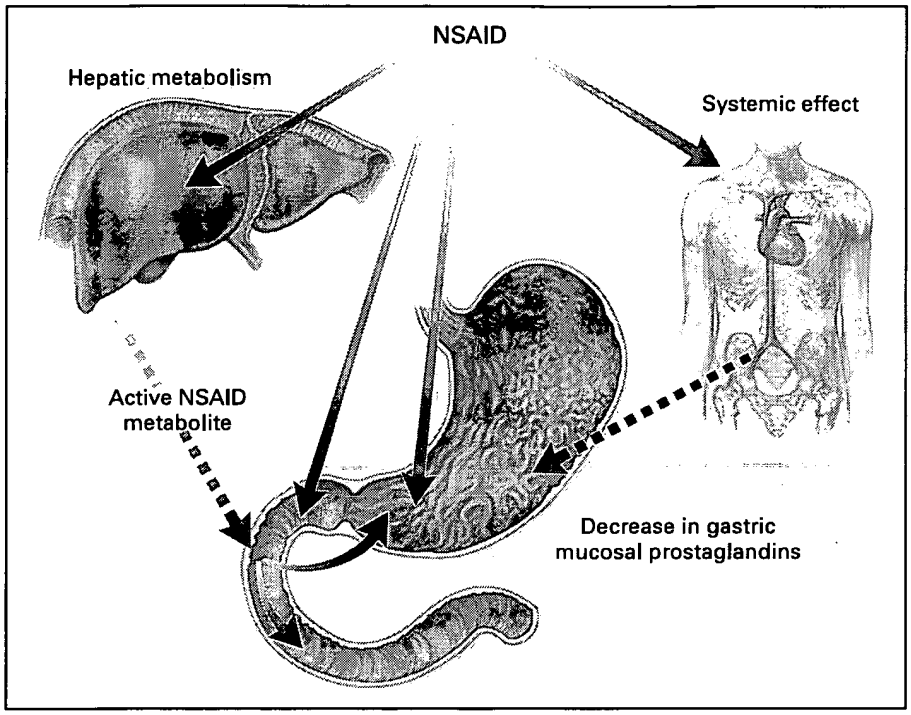

Figure 2. Mechanisms by Which NSAIDs Induce Gastroduodenal Mucosal Injury.

According to the dual-injury hypothesis of Schoen and Vender,[37] NSAIDs have direct toxic effects on the gastroduodenal mucosa (solid arrows) and indirect effects through active hepatic metabolites and decreases in mucosal prostaglandins (broken arrows). Hepatic metabolites are excreted into the bile and subsequently into the duodenum, where they cause mucosal damage to the stomach by duodenogastric reflux and mucosal damage to the small intestine by antegrade passage through the gastrointestinal tract. Adapted from Schoen and Vender.[37]

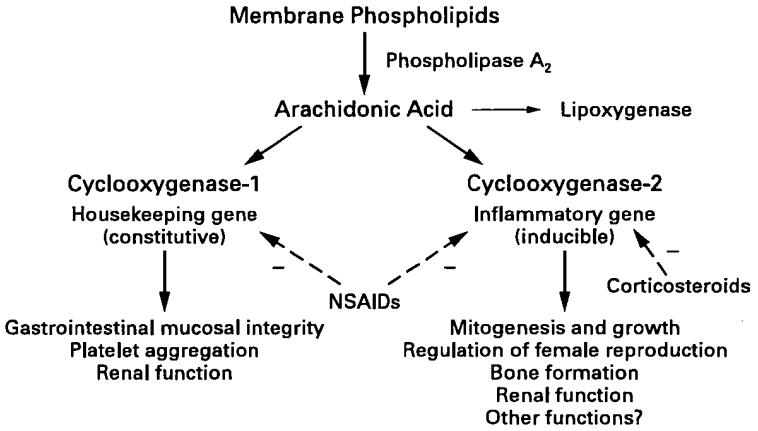

Figure 3. Biosynthesis of Prostaglandins through the Cyclooxygenase Pathways.

The immediate precursor of prostaglandins, arachidonic acid, is derived from membrane phospholipids and is catalyzed by the two cyclooxygenase isoenzymes (also designated as prostaglandin H synthase), cyclooxygenase-1 and cyclooxygenase-2. The gene for cyclooxygenase-1, the housekeeping enzyme, is expressed constitutively and maintains the homeostasis of organs, including gastric mucosal integrity. In contrast, the gene for cyclooxygenase-2, the inflammatory enzyme, is inducible. Although both pathways can be variably inhibited by different NSAIDs, only the gene for cyclooxygenase-2 contains a corticosteroid-responsive repressor element in its promoter region. The broken arrows indicate the inhibitory effects of pharmacologic agents.

by inflammatory stimuli and mitogens in many different types of tissue, including macrophages and synovial cells.[43] It has thus been suggested that the antiinflammatory properties of NSAIDs are mediated through the inhibition of cyclooxygenase-2, whereas adverse effects, such as gastroduodenal ulceration, occur as a result of effects on the constitutively expressed cyclooxygenase-1.[43,49] As discussed below, current strategies for developing NSAIDs with an improved safety profile include the selective inhibition of cyclooxygenase-2, with the sparing of cyclooxygenase-1.

Although there is substantial evidence that the suppression of gastric prostaglandins is the fundamental mechanism responsible for the gastrointestinal toxicity of NSAIDs, some studies suggest that other mechanisms may be involved. For example, ulcers do not develop spontaneously in mice with a disrupted cyclooxygenase-1 gene,[52] and Wallace et al.[53,54] reported that NSAID-induced injury occurred in association with enhanced adherence of neutrophils to the gastric vascular endothelium, as the result of an increase in the expression of intercellular adhesion molecule 1 in the basal endothelium.[55-58] Neutrophil adherence, in turn, causes mucosal injury through the release of oxygen-derived free radicals and proteases.[1]

CLINICAL SPECTRUM OF INJURY

In the majority of patients, NSAID-induced gastroduodenal mucosal injury is superficial and self-limited. However, peptic ulcers develop in some patients, and they may lead to gastroduodenal hemorrhage, perforation, and death. Serious complications of NSAID use that are less commonly recognized include pill esophagitis, small-bowel ulceration, small-bowel strictures, colonic strictures, diverticular disease, and exacerbations of inflammatory bowel disease.[9]

The spectrum of NSAID-related gastroduodenal injury includes a combination of subepithelial hemorrhages, erosions, and ulcerations that is often referred to as NSAID gastropathy. The distinction between erosions and ulcerations depends on pathological definitions, with ulcers defined as lesions that penetrate to the level of the submucosa and erosions defined as lesions confined to the mucosa. For practical purposes, an endoscopic definition is used, which is based on a subjective assessment of the size, shape, and depth of the lesion. Erosions are likely to be small and superficial, whereas ulcers tend to be larger (more than 5 mm in diameter) and deeper.[9]

After ingestion of an NSAID, ultrastructural damage to the gastric surface epithelium occurs within minutes, and gross, endoscopically detectable hemorrhages and erosions in the gastroduodenal epithelium occur within several hours.[59] However, mucosal adaptation appears to occur in response to long-term administration of aspirin in most persons.[60,61] No area of the stomach is resistant to NSAID-induced mucosal injury; the most frequently and severely affected site is the gastric antrum.[59] Although the prevalence and severity of acute injury vary according to the drug formulation,[62-64] the acute injury commonly observed during short-term administration of NSAIDs is not closely correlated with the subsequent development of the more clinically relevant process of mucosal ulceration[20,21,65,66] or with serious complications.[10,67,68] Duodenal mucosal injury occurs less commonly than gastric damage; however, ulcer complications associated with NSAIDs occur with nearly equal frequency in these two sites.[51,66] Prospective, cross-sectional endoscopic studies have shown that the combined prevalence of gastric and duodenal ulcers is 10 to 25 percent in patients with chronic arthritis treated with NSAIDs,[10,67] which is 5 to 15 times the expected prevalence in an age-matched healthy population.

TREATMENT OF NSAID-RELATED DYSPEPSIA

At least 10 to 20 percent of patients have dyspeptic symptoms during NSAID therapy.[10,11] However, such symptoms are poorly correlated with the endoscopic appearance and severity of mucosal injury, since up to 40 percent of persons with endoscopic evidence of erosive gastritis are asymptomatic,[10,68] and conversely, as many as 50 percent of patients with dyspepsia have normal-appearing mucosa.[10]

Histamine H_2-Receptor Antagonists

Several studies using different methods have shown an improvement in dyspeptic symptoms when histamine H_2-receptor antagonists are given to patients taking NSAIDs.[69-73] A recent prospective, observational cohort study by Singh et al.,[11] however, found that asymptomatic patients with rheumatoid arthritis who were taking H_2-receptor antagonists had a significantly higher risk of gastrointestinal complications than those not taking these drugs. The explanation for this surprising observation is unknown, but it might be due to the masking of dyspeptic symptoms associated with mucosal injury. Therefore, although H_2-receptor antagonists are effective in reducing NSAID-related dyspepsia, their routine use in asymptomatic patients taking NSAIDs cannot be recommended. Patients receiving H_2-receptor antagonists for the treatment of dyspepsia must be monitored carefully for the development of serious complications. The initial dose should generally be low (e.g., 400 mg of cimetidine, 150 mg of ranitidine or nizatidine, or 20 mg of famotidine, administered twice daily in each case), and the dose should be tailored to the needs of each patient.

Proton-Pump Inhibitors

In two recent studies, the proton-pump inhibitor omeprazole was compared with ranitidine[74] or mi-

soprostol,[75] a prostaglandin E_1 analogue, for the treatment and prevention of NSAID-related gastroduodenal ulcers. A secondary goal in both of these multicenter trials was to assess the effects of therapy on dyspeptic symptoms. In both studies, although different methods were used to assess the clinical response, omeprazole provided greater symptomatic relief. After four weeks, only 6 percent of patients treated with omeprazole had moderate-to-severe symptoms, as compared with 52 percent at base line, whereas 12 percent of those treated with ranitidine had such symptoms, as compared with 50 percent at base line.[74] A quality-of-life evaluation showed that the patients receiving omeprazole had significantly greater improvement in scores on the Gastrointestinal Symptom Rating Scale than the patients receiving misoprostol.[75] Because proton-pump inhibitors represent a suitable means of preventing the development of gastroduodenal ulcers associated with the use of NSAIDs,[76] they appear to provide a safe and effective form of therapy for NSAID-associated dyspepsia.

MANAGEMENT OF NSAID-RELATED GASTRODUODENAL ULCERS

The optimal treatment for patients with NSAID-induced gastroduodenal ulcers should include the elimination of any potentially aggravating factors. Nontoxic analgesics such as acetaminophen should be substituted for NSAIDs when possible, and in patients with inflammatory arthritides, disease-modifying (or slow-acting) antirheumatic drugs have been recommended as first-line treatment. If NSAID therapy is discontinued, effective treatment aimed at healing the acute ulcer can be instituted with one of several antisecretory agents or with sucralfate. If the use of NSAIDs must be continued, ulcer healing is entirely dependent on the specific agent chosen for ulcer treatment.

Mucosal Protective Agents

Sucralfate, a basic aluminum salt of sucrose octasulfate, is effective in the treatment of both NSAID-related duodenal ulcers and those unrelated to NSAIDs, and the agent appears to be as effective as H_2-receptor antagonists in the healing of non–NSAID-related gastric ulcers.[77] However, sucralfate has no proven benefit in the treatment or prevention of NSAID-related gastric ulcers. Prostaglandins exert their therapeutic effects both by enhancing mucosal defensive properties and by inhibiting gastric-acid secretion.[39] Although they are effective in preventing NSAID-induced gastroduodenal mucosal injury, their role in the treatment of NSAID-associated ulcers is unclear. Hawkey et al.[75] recently compared the capacity of misoprostol (200 μg given four times daily) and omeprazole (20 mg or 40 mg given once daily) to heal gastroduodenal ulcers in patients receiving ongoing NSAID therapy. After eight weeks of therapy, 89 percent of the patients with duodenal ulcers who received omeprazole at either dose had healing, as compared with only 77 percent of those with duodenal ulcers who received misoprostol. Among the patients with gastric ulcers, healing was detected in 80 percent of those receiving 40 mg of omeprazole, in 87 percent of those receiving 20 mg of omeprazole, and in 73 percent of those receiving misoprostol.[75]

Antisecretory Drugs

The efficacy of H_2-receptor antagonists in the treatment of NSAID-related ulcers has not been assessed extensively. Both open, uncontrolled, nonrandomized studies[78] and prospective, randomized studies[79] have suggested that treatment with conventional doses of H_2-receptor antagonists for 6 to 12 weeks results in the healing of approximately 75 percent of gastric ulcers (range, 50 to 88 percent) and 87 percent of duodenal ulcers (range, 67 to 100 percent), despite the continued use of NSAIDs. When the use of NSAIDs is continued, healing appears to be delayed and is largely dependent on the initial size of the ulcer. O'Laughlin et al.[80] reported a 90 percent healing rate for small gastric ulcers (less than 5 mm in diameter) after an eight-week course of treatment with cimetidine, whereas only 25 percent of larger ulcers healed.

In a multicenter trial that included a small subgroup of patients with NSAID-related gastric ulcers, Walan et al.[81] reported that among the patients who continued to receive NSAIDs, the healing rate was higher for those treated with omeprazole than for those treated with ranitidine. A more recent multicenter trial by Yeomans et al.[74] also demonstrated the superiority of omeprazole over ranitidine in the treatment of NSAID-related gastroduodenal ulcers. In this study, the rates of ulcer healing at eight weeks were 79, 80, and 63 percent in the groups receiving 40 mg of omeprazole, 20 mg of omeprazole, and 150 mg of ranitidine twice a day, respectively. A study by Agrawal et al.[82] compared the efficacy of lansoprazole with that of ranitidine in the healing of gastric ulcers during continued NSAID therapy. After eight weeks, ulcers were healed in 57 percent of the patients receiving 150 mg of ranitidine twice daily, whereas ulcers were healed in 73 percent of those receiving 15 mg of lansoprazole once daily and 75 percent of those receiving 30 mg of lansoprazole once daily. These observations suggest that proton-pump inhibitors can heal gastroduodenal ulcers more effectively than H_2-receptor antagonists, whether or not NSAIDs are continued.

PREVENTION OF NSAID-ASSOCIATED GASTRODUODENAL ULCERS

Because of the prevalence and severity of NSAID-related gastrointestinal complications, recent efforts

have been directed at the prevention of mucosal injury induced by NSAIDs. As discussed above, the best way to prevent mucosal injury is to avoid the use of NSAIDs and to substitute an agent less toxic to the gastroduodenal mucosa, such as acetaminophen, salsalate, or magnesium salicylate. Nevertheless, a potent NSAID is commonly preferred, and two strategies have been used to improve their safety: the administration of concomitant medication to protect the gastroduodenal mucosa from injury and the development of safer antiinflammatory agents.

Concomitant Therapy

Sucralfate

Early, small studies suggested that sucralfate might reduce gastroduodenal mucosal injury associated with the use of NSAIDs.[83] However, a large, controlled, randomized trial conducted by Agrawal et al.[84] showed no significant benefit of sucralfate in preventing gastric ulcers in patients with osteoarthritis who were receiving NSAID therapy.

H_2-Receptor Antagonists

Two large, placebo-controlled, prospective trials investigated the protective effect of ranitidine in patients with arthritis who were receiving NSAID therapy.[85,86] Ranitidine (150 mg given twice a day) was effective in preventing duodenal ulcers, which developed in 0 percent and 1.5 percent of the ranitidine-treated patients in the two studies, as compared with 8 percent of the placebo-treated patients in both studies. In contrast, the same dose of ranitidine was ineffective in preventing gastric ulcers in both studies. Taha et al.[73] recently reported a benefit of high-dose famotidine (40 mg given twice a day), as compared with placebo, in preventing both gastric and duodenal ulcers in patients with arthritis who received NSAIDs for 24 weeks. Symptomatic relief was also observed in the group randomly assigned to famotidine, but the benefit, although statistically significant, was only moderate, and the cost of such doses of H_2-receptor antagonists is considerable. Thus, the use of H_2-receptor antagonists for the prevention of NSAID-associated ulcers cannot be recommended.

Proton-Pump Inhibitors

Although proton-pump inhibitors had previously been demonstrated to heal gastroduodenal ulcers effectively in NSAID users,[81] until recently only two small studies[87,88] had systematically examined their effectiveness in preventing NSAID-related gastroduodenal mucosal injury. A recent study compared omeprazole and ranitidine for the prevention of recurrent gastroduodenal ulcers in a large number of patients with arthritis in whom NSAID therapy could not be discontinued.[74] After six months of treatment, 16.3 percent of the patients treated with ranitidine had gastric ulcers, and 4.2 percent had duodenal ulcers. In the omeprazole group, only 5.2 percent of the patients had gastric ulcers, and only 0.5 percent had duodenal ulcers.[74]

Another recent study compared omeprazole (20 mg given once a day) and misoprostol (200 μg given twice a day) for the prevention of recurrent ulcers in patients with arthritis who were receiving NSAID therapy.[75] After six months, 12 percent of the patients receiving placebo and 10 percent of those receiving misoprostol, but only 3 percent of those receiving omeprazole, had duodenal ulcers. Gastric ulcers recurred in 32 percent of the patients receiving placebo, in 10 percent of those receiving misoprostol, and in 13 percent of those receiving omeprazole.[75] These studies suggest that, like misoprostol, proton-pump inhibitors are superior to H_2-receptor antagonists. Although a prospective analysis of clinical outcomes has not been performed, these agents appear to be effective in preventing the recurrence of ulcers during continued use of NSAIDs.[76]

Prostaglandins

In their initial study, Graham et al.[67] reported that the prevalence of gastric ulcers in patients with osteoarthritis who were receiving NSAIDs was 1.4 percent in those receiving concomitant treatment with 200 μg of misoprostol four times a day, 5.6 percent in those receiving 100 μg of misoprostol four times a day, and 21.7 percent in those receiving placebo. The efficacy of misoprostol as prophylaxis against duodenal ulcers was confirmed in a subsequent study by Graham et al.[89] Despite the effectiveness of misoprostol in preventing gastroduodenal ulcers, the agent was not associated with any improvement in dyspeptic symptoms in these studies. Furthermore, diarrhea developed in many of the patients receiving the 200-μg dose of misoprostol. Raskin et al.[90] compared three regimens of misoprostol (200 μg given twice, three times, or four times a day) and concluded that although lower doses of misoprostol are better tolerated, the drug needs to be taken at least three times a day to provide effective prophylaxis against NSAID-induced gastric ulcers.

It must be emphasized that the prevention of endoscopically detectable ulcers as an end point is not necessarily a safeguard against the development of serious ulcer-related complications. To determine whether treatment with misoprostol could affect the incidence of ulcer complications caused by NSAID use, Silverstein et al.[24] conducted the Misoprostol Ulcer Complication Outcomes Safety Assessment (MUCOSA) study. They reported a 40 percent reduction in the overall rate of complications due to NSAID-associated ulcers in a group of patients receiving 200 μg of misoprostol four times a day, as compared with the patients receiving placebo.[24]

Although misoprostol is highly effective for preventing NSAID-induced ulcers and is the only drug

approved by the Food and Drug Administration as prophylaxis against NSAID-related gastroduodenal ulcers, it has a number of adverse effects. These include diarrhea and abdominal pain associated with the increased generation of cyclic adenosine monophosphate in the small intestine and increased uterine contractility that can lead to spontaneous abortion.

Development of Safer NSAIDs

Several modifications in the formulation of NSAIDs have been introduced in recent years to reduce their toxicity. Recent surveillance and endoscopic studies have confirmed that the incidence of gastroduodenal mucosal injury is reduced with the use of nabumetone, etodolac, and meloxicam.[91-93] The improved safety of meloxicam appears to be due to its preferential inhibition of cyclooxygenase-2, with a minimal effect on cyclooxygenase-1. In contrast, nabumetone and etodolac appear to inhibit cyclooxygenase-2 preferentially at low doses, but the preferential inhibition of cyclooxygenase-2 is diminished at higher doses. These agents also have other properties that contribute to their safety. Etodolac has a low level of enterohepatic recirculation and a short half-life; nabumetone is a nonacidic prodrug formulation and has no enterohepatic recirculation.[94]

Highly Selective Cyclooxygenase-2 Inhibitors

Highly selective cyclooxygenase-2 inhibitors have recently been developed that, in studies to date, have had a markedly reduced capacity to cause injury to the gastroduodenal mucosa.[95-98] Two of the compounds, celecoxib and rofecoxib, have been studied extensively, and they appear to maintain their selectivity for cyclooxygenase-2 at doses substantially higher than those required to affect inflammation. These agents are more than 100 times as selective in their ability to inhibit cyclooxygenase-2 as the currently available NSAIDs and have been shown to promote the development of gastroduodenal ulcers at a rate not significantly different from that of placebo.[99,100] The selectivity ratios for inhibition of cyclooxygenase-1 and cyclooxygenase-2 of celecoxib, rofecoxib, and other agents have been determined primarily by in vitro assays.[101] Although these drugs have similar in vivo selectivity, genetic differences among patients may affect the cyclooxygenase-2 selectivity of these drugs. Celecoxib became available for use in the United States in February 1999, and rofecoxib will probably be available very soon.

In spite of enthusiasm for these promising new NSAIDs, some questions remain regarding their highly selective inhibition of cyclooxygenase-2. For example, cyclooxygenase-2 might generate endogenous prostanoids that are biologically important (Fig. 3). Mice in which the gene for cyclooxygenase-2 has been disrupted have defects in renal function and regulation of bone resorption, and female mice have impaired reproductive physiology.[94] Mizuno et al.[102] have suggested that an increase in mucosal cyclooxygenase-2 expression may be necessary for the normal healing of gastroduodenal ulcers. However, nonselective NSAIDs also inhibit cyclooxygenase-2 to varying degrees, and the critical factor may be the ratio of isoenzyme inhibition.

McAdam et al.[103] recently reported that celecoxib, but not ibuprofen, suppressed the urinary excretion of prostacyclin in healthy subjects, whereas thromboxane activity related to cyclooxygenase-1 was suppressed only by ibuprofen. The authors speculated that long-term therapy with these agents might increase the rate of thrombotic events in patients who were at increased risk for cardiovascular disease, although no data were collected on such events.[103] On a positive note, the expression of cyclooxygenase-2 messenger RNA is enhanced in human colorectal adenomas and adenocarcinomas, and selective cyclooxygenase-2 inhibition may thereby reduce the risk of colorectal cancer.[104] The results of these studies indicate that although the highly selective cyclooxygenase-2 inhibitors offer considerable promise in the treatment of inflammatory arthritides, careful surveillance will be important to determine their ultimate benefit and safety profile.

NSAIDs Containing Nitric Oxide

Nitric oxide has a critical role in maintaining the integrity of the gastroduodenal mucosa, exerting many of the same effects as endogenous prostaglandins.[105-107] It has even been suggested that nitric oxide and prostaglandins may act synergistically to mediate mucosal protective effects,[1] and Salvemini et al.[108] have demonstrated that nitric oxide stimulates cyclooxygenase enzymes. Such redundancy in preserving normal physiologic function is not unique, and it constitutes the rationale for the development of formulations in which nitric oxide is released and compensates for the suppression of mucosal prostaglandins. Under these conditions, the desired effects of NSAIDs are maintained, including the inhibition of both cyclooxygenase isoenzymes, while toxicity is minimized (Fig. 4).[109-111] Nitric oxide–containing compounds have antiinflammatory and antipyretic activities that are similar to those of the parent compound and may have analgesic effects that are greater than those of the parent compound.[110]

In a recent seven-day clinical trial, a flurbiprofen–nitric oxide formulation was found to cause fewer gastric erosions than the parent drug, with the same inhibitory effects on gastric mucosal prostaglandin synthesis and serum thromboxane levels.[112] In addition, nitric oxide, like aspirin, inhibits platelet aggregation, but it does not suppress cyclooxygenase activity or cause gastric mucosal injury.[113] The use of nitric oxide–aspirin compounds as prophylaxis against

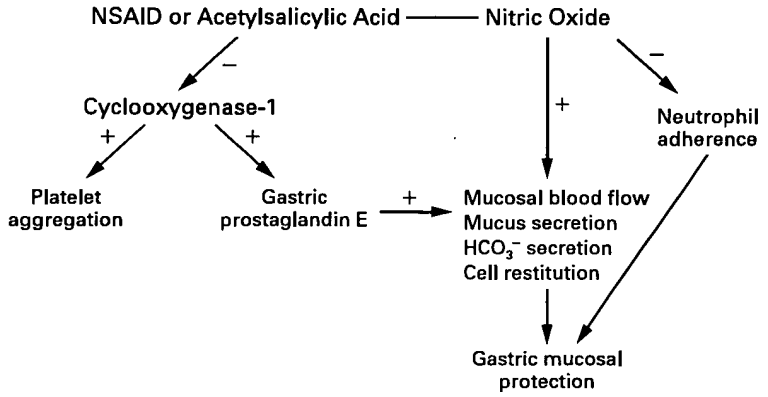

Figure 4. Postulated Mechanism by Which Nitric Oxide–Releasing NSAIDs Maintain the Ability to Protect the Gastroduodenal Mucosa while Suppressing the Level of Endogenous Mucosal Prostaglandins.

Nitric oxide appears to stimulate some of the defensive properties of the mucosa that are affected by inhibition of the cyclooxygenase-1 isoenzyme. In addition, nitric oxide inhibits intercellular adhesion molecule 1, thereby decreasing neutrophil adherence, resulting in the prevention of NSAID-induced gastroduodenal mucosal injury. Adapted from Wallace.[1]

TABLE 2. CURRENT RECOMMENDATIONS FOR THE TREATMENT OF NSAID-RELATED DYSPEPSIA AND MUCOSAL INJURY.

CLINICAL SITUATION	RECOMMENDATION
Dyspepsia	Empirical treatment with H_2-receptor antagonist (e.g., 400 mg of cimetidine, 150 mg of ranitidine or nizatidine, or 20 mg of famotidine, all twice daily) or proton-pump inhibitor (e.g., 20 mg of omeprazole, 30 mg of lansoprazole, 20 mg of rabeprazole, or 40 mg of pantoprazole daily before breakfast); individualize therapy
Helicobacter pylori infection	Treatment to eradicate infection only in patients with a history of peptic ulcer
Active gastroduodenal ulcer NSAID discontinued	Treatment with an H_2-receptor antagonist (e.g., 800 mg of cimetidine, 150 mg of ranitidine or nizatidine, or 40 mg of famotidine daily before bedtime) or a proton-pump inhibitor (as above)
NSAID continued	Treatment with a proton-pump inhibitor (as above)
Prophylactic therapy	Concomitant treatment with misoprostol ($\geq$200 μg three times a day), a proton-pump inhibitor (as above), or a cyclooxygenase-2–preferential or cyclooxygenase-2–selective NSAID

myocardial and cerebrovascular ischemia is also under investigation.

Other Approaches

Several other compounds are being developed, including NSAIDs associated with zwitterionic phospholipids, chiral NSAIDs, basic fibroblast growth factor, and trefoil peptides.[94] Although initial studies indicate that some of these compounds may help reduce the gastrointestinal toxicity of NSAIDs, their clinical use awaits further investigation.

SUMMARY

Recommendations for the prevention and management of gastroduodenal mucosal injury associated with NSAIDs are proposed in Table 2. Symptoms associated with the use of NSAIDs are common and can generally be treated empirically with an H_2-receptor antagonist or a proton-pump inhibitor. Although additional studies are necessary, eradication of *H. pylori* should be reserved for patients with a history of ulcer disease. In general, if a gastroduodenal ulcer develops, the most prudent approach is to discontinue

the NSAID and substitute therapy with acetaminophen or a nonacetylated salicylate. If treatment with the NSAID must be continued, proton-pump inhibitors should be used, since they appear to heal ulcers at the same rate, whether or not NSAID therapy is continued. After the ulcer has healed and it has been determined that NSAID therapy must be continued, the most effective prophylaxis against recurrent ulcers is the concomitant administration of misoprostol (at least 200 μg given three times a day) or a proton-pump inhibitor, or the use of an NSAID that preferentially or selectively inhibits cyclooxygenase-2. The ultimate choice of therapy in a particular patient depends on several things, including risk factors, the preferences of the patient and the physician, and cost. The development of cyclooxygenase-2–selective inhibitors and the formulation of other new, safer NSAIDs should broaden the range of options.

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CORRECTION

Gastrointestinal Toxicity of Nonsteroidal Antiinflammatory Drugs

Gastrointestinal Toxicity of Nonsteroidal Antiinflammatory Drugs . On page 1896, in Table 2, the recommendation for "Active gastroduodenal ulcer NSAID discontinued" should have read, "Treatment with an H_2-receptor antagonist (e.g., 800 mg of cimetidine, *300* mg of ranitidine or nizatidine, or 40 mg of famotidine daily before bedtime)," not "*150* mg of ranitidine or nizatidine," as printed.

NSAID-Induced Gastric Damage in Rats: Requirement for Inhibition of Both Cyclooxygenase 1 and 2

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Background & Aims: Selective cyclooxygenase (COX)-2 inhibitors produce less gastric damage than conventional nonsteroidal anti-inflammatory drugs (NSAIDs), suggesting that NSAIDs cause damage by inhibiting COX-1. We tested this hypothesis in rats by using a selective COX-1 inhibitor (SC-560). *Methods:* The effects of SC-560, celecoxib (selective COX-2 inhibitor), or a combination of both inhibitors on gastric damage and prostaglandin synthesis were determined. Selectivity of the drugs for COX-1 vs. COX-2 was assessed in the carrageenan-airpouch model. A COX-1–preferential inhibitor, ketorolac, was also evaluated. The effects of these inhibitors on leukocyte adherence to vascular endothelium and on gastric blood flow were assessed. *Results:* SC-560 markedly reduced gastric prostaglandin synthesis and platelet COX-1 activity, but spared COX-2 and did not cause gastric damage. Celecoxib did not affect gastric prostaglandin E_2 synthesis and did not cause gastric damage. However, the combination of SC-560 and celecoxib invariably caused hemorrhagic erosion formation, comparable to that seen with indomethacin. Ketorolac caused damage only at doses that inhibited both COX isoforms, or when given with a COX-2 inhibitor. Celecoxib, but not SC-560, significantly increased leukocyte adherence, whereas SC-560, but not celecoxib, reduced gastric blood flow. *Conclusions:* Inhibition of both COX-1 and COX-2 is required for NSAID-induced gastric injury in the rat.

The identification of two isoforms of cyclooxygenase (COX) has led to a reevaluation of the mechanism through which nonsteroidal anti-inflammatory drugs (NSAIDs) cause injury to the gastric mucosa. The observation that selective inhibition of COX-2 spares gastric prostaglandin (PG) synthesis and is associated with a greatly reduced incidence of gastric erosions compared with what is observed with conventional NSAIDs[1,2] has led to the hypothesis that it is the suppression of gastric COX-1 by NSAIDs that is the key mechanism responsible for erosion formation.[3–5] However, this remains an unproven hypothesis. Mice in which the gene for COX-1 is disrupted exhibit greatly reduced gastric PG synthesis, but no gastric injury.[6] Although it is possible that the lack of gastric damage in these mice is attributable to compensatory changes in mucosal defense in response to the reduced PG synthesis, one cannot rule out the possibility that reduced COX-1 activity, alone, is not sufficient for erosion formation. Indeed, when given indomethacin, a dual inhibitor of COX-1 and COX-2, these mice did develop gastric erosions.[6]

Many studies in recent years have suggested that COX-2 can contribute to gastric mucosal defense, at least in some circumstances. For example, Gretzer et al.[7] reported that selective COX-2 inhibitors interfered with adaptive response of the gastric mucosa to a topical irritant. Normally, the topical irritant increased the resistance of the gastric mucosa to damage induced by subsequent administration of a damaging agent, such as absolute ethanol. However, when pretreated with a selective COX-2 inhibitor, this protective response was inhibited, despite the fact that significant inhibition of gastric PG synthesis could not be detected.[7] COX-2 is expressed in the human stomach colonized by *Helicobacter pylori*, and it has been suggested that the PGs derived from COX-2 play a role in protecting the stomach against damage associated with this infection.[8] A role of COX-2–derived PGs in gastric ulcer healing is supported by studies in experimental models.[9–11] Because all conventional NSAIDs inhibit both COX-1 and COX-2 when administered at doses effective in reducing inflammation and pain,[5] it is possible that inhibition of both of these isozymes in the gastric mucosa contributes to the generation of erosions and ulcers.

Recently, Smith et al.[12] described the effects of a selective inhibitor of COX-1 (SC-560) in rat models of pain and inflammation. This compound was found to markedly reduce gastric PG content, but surprisingly, the effects on mucosal integrity were not mentioned. In the present study, we used this selective inhibitor of

Abbreviations used in this paper: COX, cyclooxygenase; ELISA, enzyme-linked immunosorbent assay; TX, thromboxane.

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COX-1 and selective inhibitors of COX-2 (celecoxib and DuP-697) to test the following hypothesis: suppression of both COX-1 and COX-2 is necessary for NSAID-induced gastric damage in the rat stomach. We have also performed studies with ketorolac, the most COX-1–selective of the currently marked NSAIDs,[5] to determine the relative contributions of inhibition of COX-1 vs. COX-2 to the generation of gastric damage. Finally, because both leukocyte adherence and reduced gastric blood flow have been suggested to contribute to the pathogenesis of NSAID-induced gastric damage,[13] the effects of the selective COX inhibitors on leukocyte adherence to the vascular endothelium in vivo and on gastric blood flow were examined.

Materials and Methods

Animals

Male Wistar rats weighing 175–200 g were obtained from Charles River Breeding Farms (Montreal, Quebec, Canada) and were housed in the Animal Care Facility at the University of Calgary. The rats were fed standard laboratory chow and tap water ad libitum. The rats were deprived of food, but not water, for 18–20 hours before an experiment. All experiments described below received prior approval from the Animal Care Committee of the University of Calgary and were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Selection of Doses of Test Drugs

A series of experiments was performed to establish the doses of SC-560 and celecoxib that would produce significant and selective inhibition of COX-1 and COX-2, respectively. The carrageenan-airpouch model has previously been used in our laboratory to determine the extent of suppression of COX-2 in vivo.[14] The PGE_2 that can be recovered in the inflammatory exudate is derived almost entirely from COX-2.[14] We have previously found that doses of celecoxib in the 5–45 mg/kg range significantly reduced the levels of PGE_2 in the inflammatory exudate without affecting whole blood thromboxane (TX) synthesis (an index of COX-1 activity).[15] In the present study, we used the airpouch model to examine the effects of celecoxib at a dose of 15 mg/kg, SC-560 at doses of 10–40 mg/kg, and the combination of these two drugs (same doses; n = 5–8 per group). For comparison, we also tested the effects of indomethacin (5 mg/kg), a dual inhibitor of COX-1 and COX-2. In some studies, a second selective inhibitor of COX-2, DuP-697, was used. We have previously shown that the dose used (10 mg/kg) suppressed COX-2 activity in vivo by more than 80% while having no effect on COX-1 activity.[14] Control rats were treated with vehicle (1% carboxy methylcellulose). One hour after oral administration of the test drugs or vehicle, carrageenan (2 mL of a 1% solution) was injected into the airpouch. Six hours later, the rats were anesthetized with halothane and the airpouch was carefully opened by an incision. The exudate fluid was collected for measurement of PGE_2 concentration by enzyme-linked immunosorbent assay (ELISA).[16] A 1-mL sample of blood was incubated at 37°C for 45 minutes then centrifuged (1000*g*; 10 minutes). The concentration of TXB_2 in the supernatant was measured by ELISA.[16]

Acute Gastric Damage

Groups of at least 5 rats each were given one of the following orally: celecoxib (15 mg/kg), SC-560 (20 or 40 mg/kg), the combination of these two drugs (same doses), or indomethacin (5 mg/kg). Control rats received an equal volume of the vehicle (1% carboxymethylcellulose). Three hours later, the rats were anesthetized with halothane and a blood sample was drawn from the inferior vena cava for measurement of whole blood TX synthesis, as described above. The stomach was removed and scored for hemorrhagic damage, by an observer unaware of the treatments the rats had received. The scoring involved measuring the lengths of all lesions, in millimeters, and summing the values to give an overall gastric damage score for each rat. A sample of the corpus region of the stomach was then excised, weighed, and added to a tube containing 1 mL of sodium phosphate buffer (10 mmol/L; pH 7.4). The tissue sample was minced with scissors for 30 seconds, then placed in a shaking water bath (37°C) for 20 minutes. The samples were centrifuged (9000*g*) for 1 minute, and the concentration of PGE_2 in the supernatant was determined by ELISA.[16]

Effects of Ketorolac

Of the NSAIDs presently on the market, ketorolac, shows the greatest selectivity for COX-1.[5] To further examine the importance of inhibition of COX-1 and COX-2 in the pathogenesis of NSAID-induced gastric injury, we examined the effects of a range of doses of ketorolac on gastric PGE_2 synthesis, whole blood TXB_2 synthesis (as an index of COX-1), inflammatory PGE_2 synthesis in the carrageenan-airpouch model (as an index of COX-2), and gastric damage. The experiments were performed in the same manner as described above. Ketorolac was tested at doses of 0.3, 1, 3, 10, and 30 mg/kg. Each group consisted of 5–6 rats.

To complement the studies of acute gastric damage induced by the combination of SC-560 and celecoxib, similar experiments were performed in which rats were given ketorolac at a dose that selectively inhibited COX-1 (3 mg/kg) alone or in combination with a selective COX-2 inhibitor (celecoxib at 15 mg/kg or DuP-697 at 10 mg/kg). As above, the rats were killed 3 hours later for blind scoring of the gastric damage.

The damage induced by acute administration of NSAIDs consists of erosions in the corpus region of the stomach. In contrast, chronic gastric ulcers induced by NSAIDs in humans are found primarily in the antrum. To determine if suppression of COX-1 and COX-2 is necessary for antral ulcer formation, we used the model of NSAID-induced antral ulceration originally described by Satoh et al.[17] Groups of rats (n = 5) were fasted for 20 hours, then given access to food for 2 hours. At

the end of the period of feeding, the rats were given the test drugs orally and were then fasted for another 24 hours. The rats were killed, and the stomach was examined by an observer unaware of the treatments they had received. The presence of antral ulceration and hemorrhage was noted. The test drugs used in this study were ketorolac (3 mg/kg; a dose selective for COX-1), celecoxib (15 mg/kg), the combination of ketorolac (3 mg/kg) and celecoxib (15 mg/kg), or ketorolac at a dose that suppressed both COX-1 and COX-2 (10 mg/kg).

Intravital Microscopy

The effects of celecoxib and SC-560 on leukocyte adherence to the vascular endothelium were examined using an intravital microscopy preparation, as described previously.[18] Rats (n = 5–6 per group) were anesthetized with sodium pentobarbital (65 mg/kg intraperitoneally [IP]). Images of the mesenteric microcirculation were recorded for 5 minutes after a 15-minute equilibration period. The mesentery was then superfused with bicarbonate-buffered saline containing celecoxib at 1 or 3 μmol/L, SC-560 at 0.3 or 1 μmol/L, the combination of SC-560 at 1 μmol/L and celecoxib at 3 μmol/L, indomethacin at 7 μmol/L, or buffer alone. The images were recorded for 5 minutes beginning 15, 30, 45, and 60 minutes after the start of the superfusion. The 3 μmol/L concentration of celecoxib was selected because it has been reported to be in the range of plasma concentrations required for anti-inflammatory effects in humans[19] and is considerably less than the concentration necessary for significant anti-inflammatory effects in the carrageenan-induced paw edema model.[12] SC-560 has been reported to inhibit COX-1 at a concentration of 0.3 μmol/L[12] and has been shown to inhibit intestinal PG synthesis in the rat at this concentration (Dr. W. MacNaughton, personal communication, April 2000). Leukocyte adherence was quantified on video playback in a blind manner. A leukocyte was considered adherent to the endothelium if it remained stationary for 30 seconds or more. Vessel diameter was monitored throughout the experiment using a digital caliper.[18]

To confirm the effectiveness of SC-560 in inhibiting COX-1 at the concentrations tested, and to rule out the possibility that celecoxib inhibited COX-1 in a similar setting to these experiments, the following experiment was performed. Blood was drawn from the inferior vena cava of 4 rats and distributed to tubes containing (final concentration) celecoxib (3 μmol/L), SC-560 (1 μmol/L), indomethacin (7 μmol/L), or vehicle. The samples were incubated for 45 minutes at 37°C, then centrifuged (1000*g*, 10 minutes). TXB_2 concentrations in the supernatant were measured by ELISA.

Gastric Blood Flow

Conventional NSAIDs have been shown to cause a decrease in gastric blood flow[20–22]; this has been suggested to contribute significantly to the pathogenesis of injury associated with these agents.[13] To determine if selective inhibition of COX-1 or COX-2 would result in a decrease in gastric blood flow, experiments were carried out in which gastric blood flow was measured by laser-Doppler flowmetry, as described in detail previously.[23] An ex vivo gastric chamber preparation was used.[23] The exposed stomach was bathed with 100 mmol/L hydrochloric acid throughout the experiment. A laser-Doppler probe was placed on the surface of the dorsal, corpus region of the stomach for continuous recording of blood flow.[23] After a 15-minute basal period, SC-560 (40 mg/kg), celecoxib (15 mg/kg), the combination of SC-560 and celecoxib, indomethacin (5 mg/kg), or vehicle was injected IP (n = 4–6 rats per group). Blood flow over the hour that followed was expressed as a percentage of the flow rate in the basal period.

Statistical Analysis

All data are expressed as mean ± SEM. Comparisons among groups of data were performed using a one-way analysis of variance followed by the Dunnett's Multiple Comparison test. An associated probability (*P* value) of <5% was considered significant.

Materials

SC-560 was provided by Dr. F. Degner of Boehringer-Ingelheim (Ingelheim, Germany). Celecoxib was obtained from Monsanto (St. Louis, MO), and ketorolac tromethamine was obtained from Roche Laboratories (Montreal, Quebec, Canada). The ELISA kits for PGE_2 and TXB_2 were obtained from Cayman Chemical Co. (Ann Arbor, MI). λ-Carrageenan was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from Fisher Scientific (Edmonton, Alberta, Canada).

Results

Selectivity of the Inhibitors

In the carrageenan-airpouch model, celecoxib inhibited PGE_2 synthesis by 97% but had no effect on whole blood TX synthesis (Figure 1). Thus, at this dose, celecoxib acted as a selective COX-2 inhibitor. In contrast, SC-560 caused almost complete inhibition (>95% inhibition) of whole blood TX synthesis at all doses tested (10–40 mg/kg), confirming its ability to inhibit COX-1. At the 40 mg/kg dose, SC-560 suppressed TX synthesis by 99% (Figure 1). However, SC-560 failed to significantly affect inflammatory PGE_2 synthesis, which has been shown to be almost exclusively derived from COX-2.[14] Indomethacin significantly inhibited COX-1 and COX-2, because both whole blood TX and inflammatory PGE_2 synthesis were markedly suppressed (Figure 1).

Gastric PG Synthesis

Celecoxib had no effect on PGE_2 synthesis in the normal stomach at doses in the 1–4 mg/kg range (Figure 2 shows data for the 15 mg/kg dose). SC-560 at a dose of 20 mg/kg did not significantly affect gastric PGE_2 syn-

thesis (14.2 ± 2.8 vs. 23.4 ± 3.5 pg/mg in vehicle-treated group). However, at a dose of 40 mg/kg, SC-560 significantly inhibited gastric PGE_2 synthesis by 70% (Figure 2). The combination of celecoxib (15 mg/kg) and SC-560 (40 mg/kg) caused a similar degree of inhibition of gastric PGE_2 synthesis as was seen with SC-560 alone (67%; Figure 2). Indomethacin inhibited gastric PGE_2 synthesis by 65% (Figure 2).

Because we could not detect any effect of celecoxib on gastric PG synthesis in healthy rats, possibly because any contribution of COX-2 to PG synthesis was negligible with respect to the contribution of COX-1, we examined the effects of celecoxib in a setting of increased gastric COX-2 expression. Gastric ulcers were induced by serosal application of acetic acid, as described in detail previously.[24] On the seventh day after induction of the ulcer, groups of 6 rats each received either celecoxib (15 mg/kg) or vehicle. Three hours later, the rats were killed and the stomach was removed. A sample of the gastric tissue from the ulcer margin was excised, and PGE_2 synthesis was measured as described in Materials and Methods. In the vehicle-treated rats, an average of 223 ± 36 pg/mg tissue of PGE_2 was released by the tissue sample. In celecoxib-treated rats, this was reduced by 48% (to 116 ± 23 pg/mg; $P < 0.05$).

Gastric Damage

In previous studies we observed that celecoxib at doses of 5, 15, or 45 mg/kg selectively inhibited COX-2 in vivo and did not produce any detectable gastric damage in the rat.[14] Based on those studies, we selected the 15 mg/kg dose for the present study and confirmed that it did not produce macroscopically (Figure 2) or histologically detectable gastric damage. Similarly, SC-560 at doses of 10, 20, or 40 mg/kg did not elicit damage in the stomach. Histological evaluation of gastric tissue fixed 3 hours after administration of SC-560 (40 mg/kg) confirmed the absence of any damage. When examined in a blind manner, the tissues from rats treated with SC-560 were indistinguishable from the tissues from rats treated with vehicle. In contrast, the combination of SC-560 (40 mg/kg) and celecoxib resulted in the development of gastric erosions in all 10 rats, with a mean gastric damage score that was significantly greater than seen in the other groups (Figure 2). The combination of celecoxib with the 20 mg/kg dose of SC-560 failed to cause

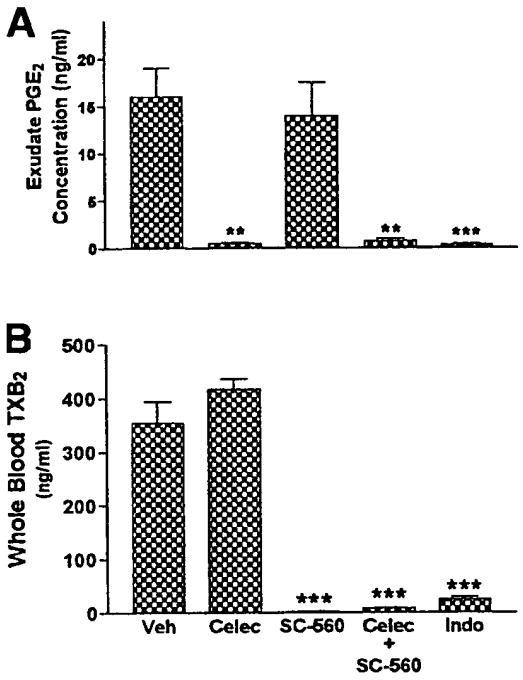

Figure 1. Effects of celecoxib (15 mg/kg) and SC-560 (40 mg/kg) on inflammatory PGE_2 synthesis (index of COX-2 activity; *A*) and whole blood TX synthesis (index of COX-1 activity; *B*). Celecoxib inhibited COX-2 but not COX-1. SC-560 inhibited COX-1 but not COX-2. The combination of celecoxib and SC-560 inhibited both COX-1 and COX-2. Indomethacin (5 mg/kg) inhibited COX-1 and COX-2 to similar extents as the two selective inhibitors. $**P < 0.01$, $***P < 0.001$ vs. vehicle-treated group. n = 5–8/group.

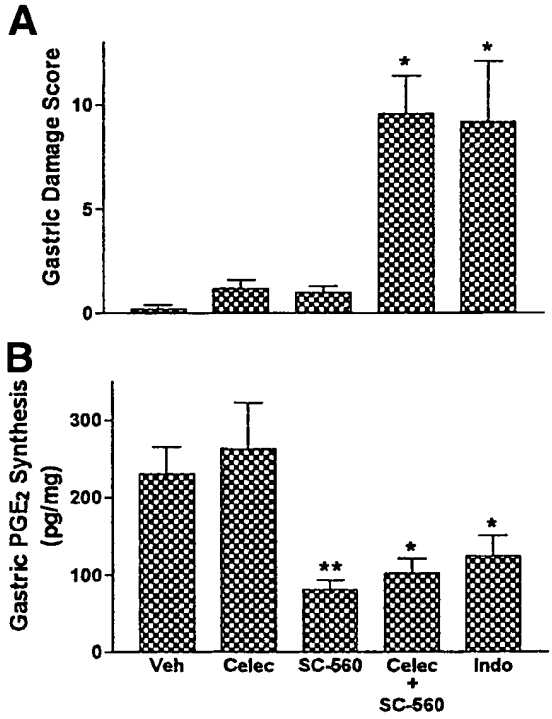

Figure 2. Effects of celecoxib (selective COX-2 inhibitor; 15 mg/kg) and SC-560 (selective COX-1 inhibitor; 40 mg/kg) on gastric mucosal integrity (gastric damage; *A*) and gastric PGE_2 synthesis (*B*). Significant increases in gastric damage were observed only in the group treated with celecoxib plus SC-560 and in the group treated with indomethacin (5 mg/kg). $*P < 0.05$, $**P < 0.01$ vs. vehicle-treated group. n = 5–10/group.

significant gastric damage (mean score, 0.2 ± 0.2). Indomethacin also caused gastric damage, the extent of which was similar to that observed with the combination of SC-560 and celecoxib (Figure 2).

Because it was possible that the increased level of damage observed in rats given both celecoxib and SC-560 was caused by topical irritant properties of celecoxib, we performed an additional experiment in which rats (n = 5 per group) were given SC-560 orally at 40 mg/kg, and celecoxib (15 mg/kg) or vehicle IP. Damage was scored 3 hours later, as in the studies described above. In the rats receiving SC-560 orally and vehicle IP, the mean damage score was 0.2 ± 0.2. In contrast, the rats that received oral SC-560 and IP celecoxib had a mean gastric damage score of 7.4 ± 1.6 ($P < 0.05$).

Effects of Ketorolac and DuP-697

As shown in Figure 3, all doses of ketorolac tested produced significant inhibition of COX-1 activity (TX synthesis) and gastric PG synthesis. Doses of ≥1 mg/kg inhibited COX-1 activity by 95% and gastric PG synthesis by >88%. Despite this, significant gastric damage was not observed with ketorolac at doses in the 0.3–3 mg/kg range. Ketorolac did not significantly affect COX-2 activity (inflammatory PG synthesis in the air-pouch model) at doses of ≤3 mg/kg. With doses of 10 and 30 mg/kg, ketorolac produced significant inhibition of COX-2 activity (by 75% and 91%, respectively) and produced significant gastric damage.

Having identified a dose of ketorolac that inhibited COX-1 but not COX-2, we then tested the effects of the

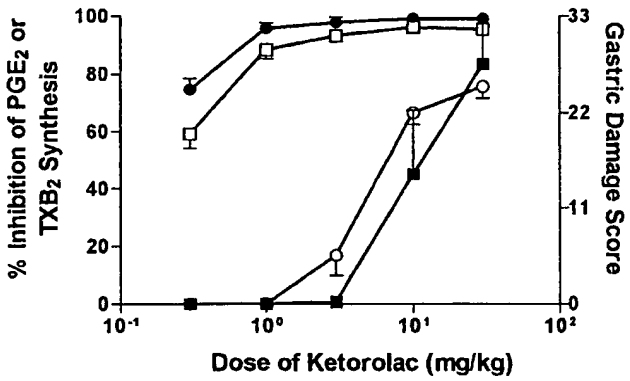

Figure 3. Inhibition of eicosanoid synthesis and gastric damaging effects of ketorolac. Effects on COX-1 activity (●; whole blood TX synthesis), COX-2 activity (○; inflammatory PGE_2 synthesis in the carrageenan-airpouch model), gastric PGE_2 synthesis (□), and gastric damage with various doses of ketorolac (■; 0.3–30 mg/kg) were assessed. Gastric PGE_2 synthesis and COX-1 activity were significantly ($P < 0.01$) suppressed at all doses of ketorolac. COX-2 activity was significantly suppressed ($P < 0.05$) only at the 10 and 30 mg/kg doses of ketorolac, the same doses that caused significant gastric damage (compared with a vehicle-treated group). Each group consisted of 6 rats.

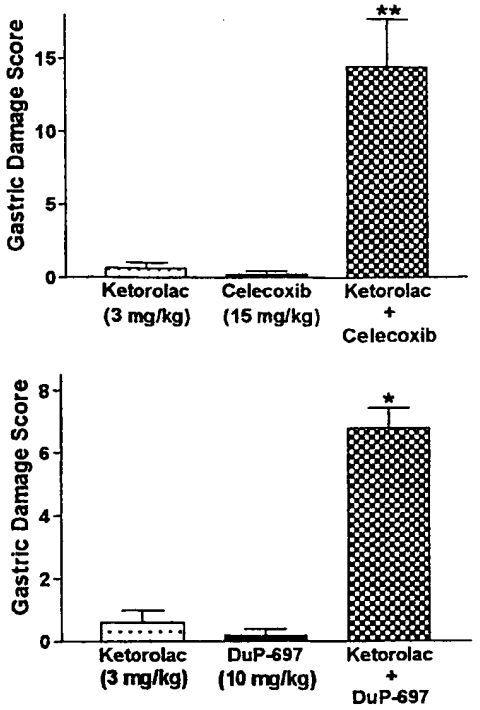

Figure 4. Gastric damaging effects of ketorolac, at a dose selectively inhibiting COX-1, DuP-697 (a selective COX-2 inhibitor), celecoxib (a selective COX-2 inhibitor), and the combination of ketorolac and one of the two COX-2 inhibitors. *$P < 0.05$, **$P < 0.01$ compared with groups treated with only one of the test drugs. n = 5–6/group.

combination of that dose of ketorolac with a selective COX-2 inhibitor. As shown in Figure 4, administration of ketorolac together with celecoxib resulted in significant gastric damage. Moreover, administration of ketorolac with DuP-697, another selective COX-2 inhibitor,[14] also resulted in significant gastric damage. DuP-697 alone failed to cause gastric damage.

We also evaluated the effects of selective inhibitors of COX-1 and COX-2 in a model in which ulceration of the antrum of the stomach is produced. Using this "refeeding" model,[17] rats given celecoxib alone (15 mg/kg) or ketorolac alone (3 mg/kg) did not exhibit any detectable damage. However, 4 of 5 rats given the combination of these two drugs developed antral damage and overt hemorrhage. Moreover, all 5 rats given a higher dose of ketorolac (10 mg/kg), which inhibits both COX-1 and COX-2 (Figure 3), developed hemorrhagic antral ulceration.

Effects of COX Inhibitors on Leukocyte Adherence

Celecoxib (3 μmol/L) caused a 6-fold increase in leukocyte adherence over basal levels (Figure 5). A significant increase in leukocyte adherence was evident within 30 minutes of beginning the superfusion of the

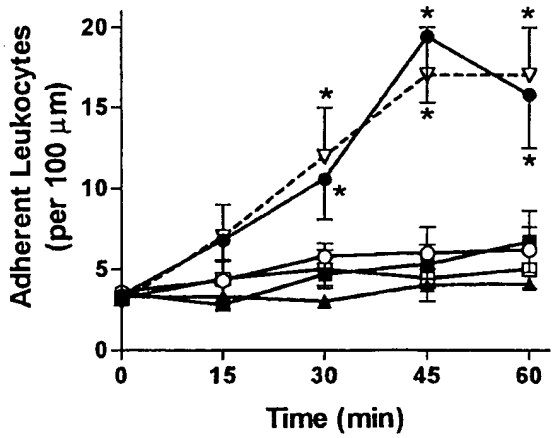

Figure 5. Effects of 1 μmol/L (○) and 3 μmol/L (●) celecoxib (selective COX-2 inhibitor), 0.3 μmol/L (□) and 1 μmol/L (■) SC-560 (selective COX-1 inhibitor), and 7 μmol/L (▽) indomethacin (dual COX-1 and COX-2 inhibitor) on leukocyte adherence to the vascular endothelium of mesenteric venules in the rat. After a basal period, the vessels were superfused with one of these inhibitors, with vehicle (▲), or with indomethacin, for a period of 60 minutes. Indomethacin (7 μmol/L) and celecoxib (3 μmol/L) significantly increased the numbers of adherent leukocytes. A combination of SC-560 and celecoxib resulted in increases in leukocyte adherence similar to that seen with SC-560 alone. *$P < 0.05$ vs. vehicle-treated group. n = 5–6/group.

blood vessels. The lower concentration of celecoxib (1 μmol/L) did not produce a statistically significant change in leukocyte adherence. SC-560 did not cause an increase in leukocyte adherence at either concentration tested (0.3 or 1 μmol/L). Indomethacin (7 μmol/L) caused adherence similar in magnitude to that seen with the higher concentration of celecoxib. Superfusion with the combination of SC-560 (1 μmol/L) and celecoxib (3 μmol/L) caused leukocyte adherence similar in magnitude to that seen with celecoxib alone.

None of the test drugs significantly affected the diameter of the mesenteric venules during the 60-minute exposure period. The mean diameter at the beginning of the experiment in all groups ranged between 30 and 35 μm. At the end of the 60-minute exposure to one of the test drugs of the vehicle, the mean diameters in the various groups ranged, as a percentage of the starting diameter, from 97% to 105% (no significant differences among the groups and no significant change from the respective basal value). Confirmation that the 3 μmol/L concentration of celecoxib did not inhibit COX-1 activity was provided by the experiments in which whole blood was exposed to this concentration of celecoxib for 45 minutes, and the generation of TXB_2 was then measured. TXB_2 synthesis in this group averaged 7.04 ± 0.10 ng · mL^{-1} · min^{-1}, compared with 5.24 ± 0.11 ng · mL^{-1} · min^{-1} in whole blood to which only the vehicle was added. On the other hand, SC-560 at a concentration of 1 μmol/L inhibited TXB_2 synthesis by 98% (0.09 ± 0.02 ng · mL^{-1} · min^{-1}). Exposure of whole blood to indomethacin (7 μmol/L) also resulted in profound inhibition of TXB_2 synthesis (0.13 ± 0.04 ng · mL^{-1} · min^{-1}; 98% inhibition).

Effects on Gastric Blood Flow

IP administration of vehicle did not cause any significant changes in gastric blood flow over the following 60-minute period (Figure 6). Similarly, administration of celecoxib (15 mg/kg IP) did not produce any significant changes in gastric blood flow. The mean rate of gastric blood flow 60 minutes after celecoxib administration was 101% ± 12% of the basal flow rate. In contrast, administration of SC-560 (40 mg/kg IP) or indomethacin (5 mg/kg) resulted in a profound reduction in gastric blood flow. In both cases, blood flow had decreased to ~50% of basal levels by 1 hour after administration ($P < 0.01$). Coadministration of SC-560 (40 mg/kg) and celecoxib (15 mg/kg) resulted in a decrease in gastric blood flow comparable with that seen with SC-560 alone. By 1 hour after administration of the drugs, gastric blood flow had decreased to 52% ± 4% of basal levels.

Discussion

Selective inhibition of COX-2 has been shown to be associated with significantly less gastric erosion formation than that seen with anti-inflammatory doses of

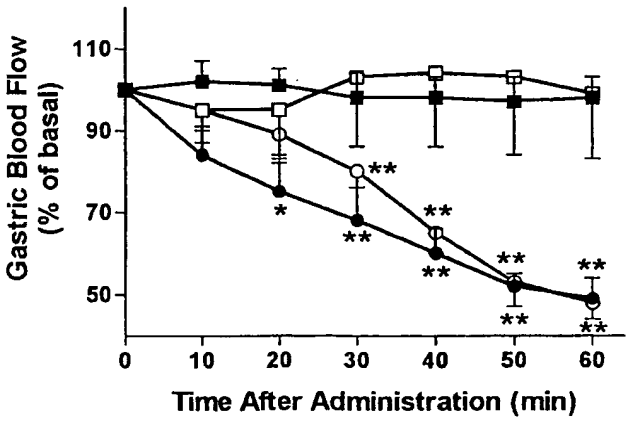

Figure 6. Effects of celecoxib (□; selective COX-2 inhibitor; 15 mg/kg IP), SC-560 (●; selective COX-1 inhibitor; 40 mg/kg IP), or indomethacin (○; 5 mg/kg IP) on gastric blood flow in the rat. After a basal period, one of the inhibitors or vehicle (■) was injected and blood flow was continuously measured by laser-Doppler flowmetry for 60 minutes. The combination of SC-560 and celecoxib resulted in decreases in gastric blood flow similar to that seen with SC-560 alone. The gastric blood flow data are expressed as a percent of the basal flow in each rat. *$P < 0.05$, **$P < 0.01$ vs. vehicle-treated group. n = 4–6/group.

conventional NSAIDs, both in animals[1,2] and humans.[4,25] Such data are consistent with the notion that the suppression of COX-1 by conventional NSAIDs underlies their ability to cause gastric damage. However, it is a presumption that blockade of COX-1 is all that is needed for erosions to develop, because before the present study, the effects of a selective COX-1 inhibitor on gastric mucosal integrity have not been reported. Even excluding the potential contribution of topical irritant properties of NSAIDs, it remained possible that the ability of these agents to inhibit COX-2 activity may also be an important component of the mechanism of action in terms of inducing gastric damage. The results of the present study suggest that inhibition of both COX-1 and COX-2 is required for the development of gastric erosions after NSAID administration in the rat. Neither a COX-1 inhibitor nor a COX-2 inhibitor caused macroscopically or histologically detectable gastric damage when given at doses that were proven to be effective at selectively inhibiting the target enzyme in vivo. However, administration of both inhibitors invariably resulted in gastric erosion development. Moreover, a COX-1–preferential inhibitor, ketorolac, was shown to cause gastric damage only when given doses in which significant inhibition of COX-2 occurred. These doses were 10–30-fold greater than the doses required for near-complete inhibition of COX-1 and gastric PG synthesis. Ketorolac (at a dose that selectively blocked COX-1) given together with DuP-697 (another selective COX-2 inhibitor) produced significant gastric damage, whereas neither drug alone caused damage. Likewise, the combination of ketorolac and celecoxib produced significant gastric damage (in the corpus and antrum), whereas neither drug alone caused significant damage.

The results of the present study also show that there are distinct mechanisms through which inhibition of COX-1 vs. COX-2 could contribute to erosion formation. We have previously proposed that NSAID-induced adherence of neutrophils to the vascular endothelium within the gastric microcirculation contributes to the generation of mucosal injury.[18,26,27] The induction of neutrophil adherence by NSAIDs is likely to be in part caused by suppression of the tonic production of PGs (such as prostacyclin) by the vascular endothelium.[28] Given the numerous reports that COX-1 is the constitutively expressed isoform of COX, responsible for "housekeeping functions,"[1–3] it was our presumption that the ability of NSAIDs to stimulate neutrophil adherence was caused by suppression of COX-1 in the vascular endothelium. The results of the present study suggest that this is not the case. Indeed, the selective COX-2 inhibitor, celecoxib, elicited significant leukocyte adherence in mesenteric venules that was comparable with that achieved with a conventional NSAID (indomethacin), whereas the selective COX-1 inhibitor (SC-560) did not. In the latter case, >98% suppression of COX-1 was confirmed through measurement of whole blood TX synthesis. Whether the effect of celecoxib is caused by suppression of COX-2 in the endothelium or in another cell type is not clear. Interestingly, however, the possibility that COX-2 is a major source of prostacyclin synthesis is supported by a recent human study in which >80% of prostacyclin synthesis in healthy volunteers could by inhibited by celecoxib.[19] Significant inhibition of prostacyclin synthesis by therapeutic doses of rofecoxib has also been shown.[29]

A decrease in gastric blood flow after NSAID administration has been documented in laboratory animals and humans,[20–22,30] and has been suggested to contribute significantly to the pathogenesis of mucosal injury.[13] The demonstration that SC-560, but not celecoxib, produced a decrease in gastric blood flow in the rat strongly suggests that the effect of NSAIDs is caused by suppression of COX-1. Both the time course and magnitude of the decrease in gastric blood flow observed with SC-560 were similar to what occurs with indomethacin and what we have previously observed with diclofenac.[30]

The conclusion that suppression of both COX-1 and COX-2 is necessary for NSAID-induced gastric damage in the rat is consistent with a number of previous findings. For example, mice in which the gene for COX-1 was disrupted, so that they did not have functional COX-1, did not exhibit spontaneous gastric damage despite negligible gastric PG synthesis. However, these mice did develop erosions when given indomethacin (a dual COX-1/COX-2 inhibitor). Warner et al.[5] recently evaluated more than 40 NSAIDs, comparing their effects on the two COX isoforms using a human whole blood assay. While they pointed to the suppression of COX-1 as a key to gastric toxicity of NSAIDs, which we do not dispute, they also showed that when tested in whole blood at concentrations that suppressed COX-2 activity by 80%, all of the conventional NSAIDs also markedly suppressed COX-1 activity (i.e., by more than 60%). Thus, despite the fact that several drugs (including ketorolac) exhibited considerable selectivity for COX-1 in vitro, these drugs would still act as nonselective inhibitors when used at doses that are effective for reducing pain and inflammation. The only other study we are aware of in which a selective COX-1 inhibitor was evaluated was that of Smith et al.[12] They showed that SC-560 could almost completely abolish gastric PG con-

tent, but conspicuous by its absence was any mention of the effects of this drug on gastric mucosal integrity. In that study, doses of SC-560 as high as 100 mg/kg were used.

As previously suggested by others,[7] our results are consistent with the hypothesis that PGs derived from COX-2 contribute to mucosal defense. However, we were not able to detect any greater reduction of gastric PGE_2 synthesis when both a COX-1 and COX-2 inhibitor were given vs. the effect of the COX-1 inhibitor alone. This is consistent with the findings of Gretzer et al.,[7] who observed an effect of several COX-2 inhibitors on mucosal resistance to injury, but could not detect any significant change in gastric PG synthesis. The most likely explanation for these findings is that the contribution of COX-2 to total PG synthesis in the normal stomach is very small (not detectable using the assay system we used, which is a measure of PG synthetic capacity), but is nevertheless very important in terms of mucosal defense. This explanation is supported by the demonstrated ability of celecoxib to inhibit PG synthesis in the stomach in a model (acetic acid–induced gastric ulcer) in which COX-2 expression is markedly up-regulated.[8]

In this study we focused on the suppression of COX activity by NSAIDs as a mechanism of gastric injury. Of course, NSAIDs have other actions unrelated to suppression of COX that contribute to damage. For example, many NSAIDs exert topical irritant effects that can contribute to mucosal injury.[31] Indeed, it was possible that topical irritant properties of celecoxib, together with the suppression of gastric PG synthesis by SC-560, accounted for the damage we observed when both drugs were given. However, IP administration of celecoxib together with oral administration of SC-560 still produced mucosal damage, suggesting that the inhibition of COX-2 by celecoxib, rather than topical irritant properties, accounted for the damage induced when the two compounds were given together. Similarly, the combination of orally administered ketorolac (at dose specific for COX-1) and IP DuP-697 (at dose specific for COX-2) caused significantly more gastric damage than either drug alone. As was the case with oral administration, the extent of damage observed with the combination of a COX-1 inhibitor and a COX-2 inhibitor was increased in a synergistic, rather than additive, manner.

In summary, this study shows that selective inhibition of either COX-1 or COX-2 does not elicit gastric damage in the rat; rather, inhibition of both isoforms of COX is required for NSAID-induced damage to develop. The results obtained with SC-560 and celecoxib were confirmed with other inhibitors of COX-1 (ketorolac) and COX-2 (DuP-697). Both COX isoforms seem to contribute to mucosal defense. We have identified distinct mechanisms through which inhibition of these isoforms may contribute to the pathogenesis of NSAID-induced gastric damage. COX-1 inhibition results in reduced gastric blood flow, whereas COX-2 inhibition leads to increased leukocyte adherence to the vascular endothelium. These results suggest that the concept that inhibition of COX-1 alone is the mechanism underlying NSAID-induced gastric injury is incorrect. Although the notion that anti-inflammatory drugs that are COX-1 sparing[32] will be less toxic to the stomach may be accurate, this term may be misconstrued such that an important contribution of COX-2 to gastric mucosal defense is overlooked.

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Prostaglandin Synthase 1 Gene Disruption in Mice Reduces Arachidonic Acid-Induced Inflammation and Indomethacin-Induced Gastric Ulceration

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Summary

Cyclooxygenases 1 and 2 (COX-1 and COX-2) are key enzymes in prostaglandin biosynthesis and the target enzymes for the widely used nonsteroidal anti-inflammatory drugs. To study the physiological roles of the individual isoforms, we have disrupted the mouse *Ptgs1* gene encoding COX-1. Homozygous *Ptgs1* mutant mice survive well, have no gastric pathology, and show less indomethacin-induced gastric ulceration than wild-type mice, even though their gastric prostaglandin E_2 levels are about 1% of wild type. The homozygous mutant mice have reduced platelet aggregation and a decreased inflammatory response to arachidonic acid, but not to tetradecanoyl phorbol acetate. *Ptgs1* homozygous mutant females mated to homozygous mutant males produce few live offspring. COX-1-deficient mice provide a useful model to distinguish the physiological roles of COX-1 and COX-2.

Introduction

The cyclooxygenase isoforms, COX-1 and COX-2, catalyze the key step in the synthesis of prostaglandins. While COX-1 and COX-2 catalyze the same reaction, the conversion of arachidonic acid (AA) to prostaglandin H_2 (PGH_2), the isoforms appear to have different biological roles. COX-1 and COX-2 are also the target enzymes for the most widely used drugs in human medicine, nonsteroidal anti-inflammatory drugs (NSAIDs). Thus, there is considerable interest in understanding the physiological roles of COX-1 and COX-2 and in developing drugs that differentially inhibit them.

The COX isoforms are encoded by genes located on different chromosomes (Wen et al., 1993). The gene encoding COX-1 (*Ptgs1* in the mouse) spans about 22 kb (Yokoyama and Tanabe, 1989; Kraemer et al., 1992), from which a message of 2.8 kb is derived (DeWitt and Smith, 1988; Merlie et al., 1988; Yokoyama et al., 1988). The gene encoding COX-2 (*Ptgs2* in the mouse) is about 8 kb (Xie et al., 1991; Kujubu et al., 1991; O'Banion et al., 1991) and produces a message of about 4.1 kb (Xie et al., 1991; Kujubu et al., 1991; O'Banion et al., 1991; Kujubu and Herschman, 1992; DuBois et al., 1994b). The COX-1 and COX-2 proteins from the same species are about 60% identical with the catalytic regions being conserved.

The genes encoding COX-1 and COX-2 differ in their regulation at the transcriptional level, and recent data reinforce the likelihood that the isoforms mediate different biological functions (Morita et al., 1995; Murakami et al., 1994). COX-1 appears to be constitutively synthesized in many tissues (Simmons et al., 1991; O'Neill and Ford-Hutchinson, 1993; Seibert et al., 1994), although its level of expression can vary with the state of differentiation or following cytokine or tumor promoter stimulation (Smith et al., 1993, 1994; Samet et al., 1995; Murakami et al., 1995). COX-1 is thought to carry out primarily "housekeeping" functions such as cytoprotection of the gastric mucosa, regulation of renal blood flow, and platelet aggregation (DeWitt and Smith, 1988, 1990; Merlie et al., 1988; Funk et al., 1991). In contrast, COX-2 message and protein are normally undetectable in most tissues; however, COX-2 expression in certain cell types can be rapidly induced by proinflammatory or mitogenic agents, including cytokines, endotoxins, tumor promoters, and mitogens (Xie et al., 1991; O'Banion et al., 1992; Hla and Neilson, 1992; Fletcher et al., 1992; DuBois et al., 1994a; Smith et al., 1994). Because of this rapid induction, the gene encoding COX-2 has been termed an immediate-early response or primary response gene (Simmons et al., 1989; Maier et al., 1990; Fletcher et al., 1992; Ryseck et al., 1992). COX-2 message and protein have been shown to be up-regulated in human colon cancers (Eberhart et al., 1994; Kargman et al., 1995) and in mouse skin papillomas and carcinomas (Muller-Decker et al., 1995).

The COX isoforms are the primary target enzymes for NSAIDs, which act by inhibiting the activity of the enzymes (Vane, 1971; Smith and Willis, 1971; Smith et al., 1990; Xie et al., 1992; Seibert et al., 1994; Masferrer et al., 1994; Mitchell et al., 1994; Seibert and Masferrer, 1994). Aspirin, the most common and best-studied NSAID, was originally shown to inhibit prostaglandin synthesis by Vane (1971). NSAIDs in common use today include aspirin, ibuprofen, and indomethacin, and all inhibit the COX enzymes. In addition to the use of NSAIDs as analgesics and for alleviation of acute and chronic inflammatory diseases such as arthritis (Levi and Shaw Smith, 1994; Simon, 1994), NSAIDs (in particular, aspirin) have proven effective for decreasing the frequency of heart attacks and strokes (Vane and Botting, 1992) and in reducing the incidence of colon cancer (Thun et al., 1991, 1993; Marnett, 1992). Some NSAIDs also inhibit chemically induced colon cancer in rodents (Rao et al., 1995, and references therein).

From the combined evidence of NSAID effects on rodent and human colon cancers, it has been suggested that randomized prevention trials with humans be initiated (Heath et al., 1994). However, while NSAIDs have many beneficial effects, they can also cause adverse side effects, the most common of which are gastrointestinal ulceration and nephrotoxicity (Clive and Stoff, 1984; Black, 1986; Brooks and Day, 1991; Price and Fletcher, 1990; Simon, 1994).

Since the discovery of COX-2, the identification of drugs that selectively inhibit this isoform has become the focus of NSAID development (Xie et al., 1992; Meade et al., 1993; Seibert and Masferrer, 1994; Mitchell et al., 1994; Masferrer et al., 1994). The rationale for this is that COX-1 is not elevated during inflammation and that, as a necessary housekeeping gene, its inhibition by NSAIDs may be the cause of their adverse side effects. In contrast, COX-2 is normally nondetectable in most tissues, but is rapidly elevated during inflammation (Masferrer et al., 1994; Crofford et al., 1994; Vane et al., 1994; Mitchell et al., 1994; Seibert et al., 1994; Vane, 1994; Simmons et al., 1991), and its inhibition by NSAIDs is thought responsible for their therapeutic effects.

The relative biological contributions of the COX-1 and COX-2 isoforms in the maintenance of normal physiological functions and in diseased states is not, however, entirely clear. Most of the current knowledge has come from studies of NSAID inhibition, COX-2 induction, or both. As an alternative approach to understand the roles of these enzymes better, we have used gene targeting to generate a mouse strain that is unable to synthesize COX-1. The development of COX-2-deficient mice is reported by Morham et al. (1995 [this issue of *Cell*]). With these mice, we hope to learn more about the roles of the two isoforms in normal physiology and in various inflammatory disorders and to understand better the etiology of the therapeutic effects and deleterious side effects of NSAIDs. In the present study, we report the generation of mice lacking COX-1 and some of their phenotypic characteristics.

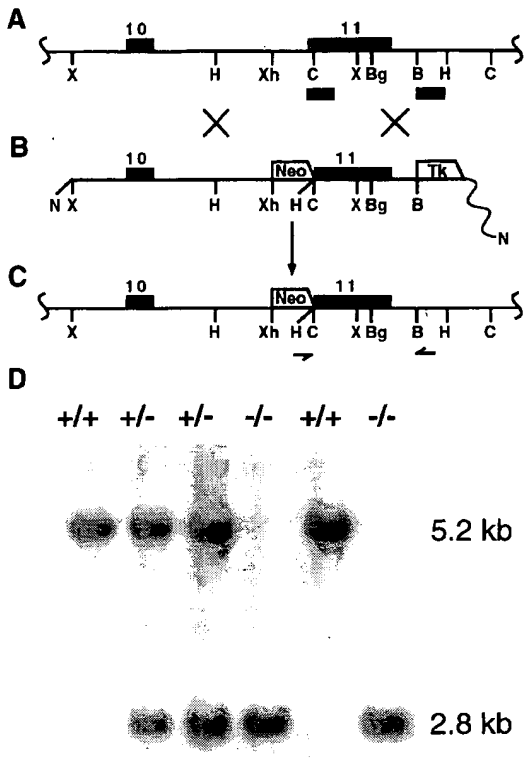

Figure 1. Targeted Disruption of the Mouse *Ptgs1* Gene

(A) The 3′ region of the *Ptgs1* gene. Closed boxes represent exons, and bars below represent the probes. Restriction sites: Bg, BglII; B, BamHI; C, ClaI; H, HindIII; X, XbaI; Xh, XhoI.

(B) Targeting construct. Phosphoglycerolkinase-promoted neomycin and herpes simplex thymidine kinase genes are represented by Neo and Tk, respectively. Wavy lines represent plasmid (not to scale). N shows the linearizing NotI restriction site.

(C) Targeted *Ptgs1* locus. Diagnostic PCR primers are indicated by arrows.

(D) Southern blot of HindIII-digested targeted allele (2.8 kb) and wild-type allele (5.2 kb) mouse tail DNA. Wild type (+/+), heterozygotes (+/−), and homozygote mutants (−/−) are shown.

Results

Cloning of the 3′ Region of the *Ptgs1* Gene

The 3′ region of the mouse *Ptgs1* gene was cloned using a 357 bp probe synthesized from strain 129 mouse embryonic stem (ES) cell DNA. The probe was made by polymerase chain reaction (PCR) with primers for the 5′ end of exon 11, the sequences of which were based on the human *COX-1* gene structure (Yokoyama and Tanabe, 1989) and the mouse cDNA sequence (DeWitt et al., 1990). This probe detected only a single band on Southern blots of mouse ES cell DNA digested with XbaI, HindIII, BglII, BamHI, or SacI, indicating that it was specific for a single gene. The 15 kb fragment cloned was subsequently shown to be from *Ptgs1*, and not the isoform *Ptgs2*, by the following. First, three different primer pairs specific for sequences in exon 11 of *Ptgs1* were used for PCR, and the predicted product sizes were obtained. Second, about 100 bp of DNA 3′ of the ClaI site in exon 11 (Figure 1A) was sequenced from the fragment and shown to match the published sequence of *Ptgs1* (DeWitt and Smith, 1990). Third, PCR with primers specific for the 5′ and 3′ ends of *Ptgs1* exon 10 produced the correct sized product from the cloned fragment. Fourth, the 2.4 kb PCR fragment used to diagnose targeted clones (Figure 1C) was digested by BglII into fragments of the predicted sizes.

Vector Construction and Targeting

The targeting strategy for disruption of the *Ptgs1* gene is shown in Figure 1. Since aspirin inactivates COX-1 by acetylating Ser-530 (Humes et al., 1981; Roth et al., 1983; DeWitt et al., 1990), we disrupted the gene prior to the codon for this amino acid in exon 11. The targeting vector was designed to replace about 1 kb of intron 10, together with the splice junction and first 44 bp of exon 11, with the neomycin resistance (*Neo*) gene (Figure 1B). If a pro-

tein were made from the resulting disrupted gene, it would lack the carboxy-terminal 120 amino acids, including Ser-530. Alternate splicing to eliminate the *Neo* gene would result in the elimination of 14 amino acids and loss of proper reading frame.

The plasmid was linearized with NotI and electroporated into strain 129–derived E14TG2a ES cells. Following electroporation, G418 and ganciclovir selection was started (Mansour et al., 1988), and 6 of 96 doubly resistant colonies isolated were positive for the expected 2.4 kb PCR product indicated in Figure 1C. Using the exon 11 probe indicated in Figure 1A, we confirmed targeting in these PCR-positive clones by detection on Southern blots of the expected 2.8 kb HindIII fragment. The same 2.8 kb band was also detected with a 510 bp BamHI–HindIII fragment that hybridizes to a genomic region 3′ to the targeting construct (Figure 1A).

Production of Animals

Cells from two of the targeted clones were injected into C57BL/6J (B6) blastocysts, resulting in the birth of four male chimeras. One chimera produced heterozygous F1 129/B6 offspring after mating to B6 females. From the first seven F2 litters obtained by mating F1 heterozygous siblings, 14 wild-type, 31 heterozygous, and 16 homozygous mutant weanling pups were obtained, in agreement with Mendelian expectations ($p > 0.9$). Southern blots of HindIII-digested tail DNA from wild-type F2, heterozygous F2, and homozygous mutant F2 mice are shown in Figure 1D.

General Health of the COX-1-Deficient Mice

The COX-1-deficient mice develop normally and appear healthy. Necropsy and microscopic examination of selected tissues (liver, spleen, kidney, gastrointestinal tract, reproductive tract, heart, and lungs) from three homozygous mutant males and three homozygous mutant females, aged 2–5 months, revealed no significant pathology. However, in three of six kidneys examined from homozygous mutant mice, a minimal change was present, characterized by one or two small foci per section of basophilic, immature tubules. The size and frequency of these lesions did not change with age. All six wild-type age-matched controls did not show these foci. There were no consistent or remarkable findings in other tissues examined, including the glandular and nonglandular stomach.

Northern Blot Analysis of the COX-1 Message

The effect of *Ptgs1* gene disruption on *COX-1* mRNA was analyzed by determining the level of message in the colons and kidneys of wild-type, heterozygous, and homozygous mutant F2 mice using the 357 bp probe described above. Figure 2A shows that the level of full-length (2.8 kb) message is reduced to approximately half normal in the heterozygous mice. No 2.8 kb message is detected in the homozygous mutant mice. Similar results (data not shown) were obtained when the 1.7 kb fragment of mouse cDNA (Oxford Biomedical) was used as the probe. When the blots were rehybridized to a probe specific for the *Neo* gene,

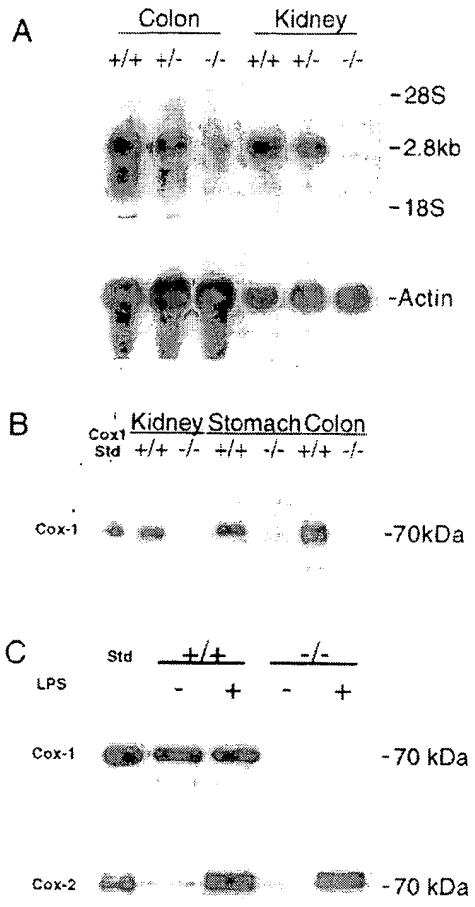

Figure 2. Northern and Western Blot Analyses

(A) Northern blot of total RNA from colon and kidney of wild-type (+/+), heterozygous (+/−), and homozygous mutant (−/−) mice. The blot was probed with a 357 bp PCR fragment from the *COX-1* cDNA.

(B) Western blot of microsomal protein from kidney, stomach, and colon of wild-type (+/+) and homozygous mutant (−/−) mice. The COX-1 standard is in the left lane. The protein was detected with a polyclonal antibody to COX-1.

(C) Western blot of total protein from control and LPS-stimulated peritoneal macrophages from wild-type (+/+) and homozygous mutant (−/−) mice. (Top) COX-1 levels in control and LPS-stimulated peritoneal macrophages from wild-type and homozygous mutant mice; a COX-1 standard is on the left; the protein was detected with an antibody to COX-1. (Bottom) COX-2 levels in control and LPS-stimulated peritoneal macrophages from wild-type and homozygous mutant mice; a COX-2 standard is on the left; the protein was detected with a polyclonal antibody to COX-2.

a band at 1.1 kb was detected (data not shown) in the heterozygous and homozygous mutant RNA samples; as expected, the band was more intense in the homozygous mutant sample. The *Neo* probe did not hybridize with any band detected by the *COX-1* probes.

Western Blot Analysis of the COX-1 Protein

A polyclonal antibody against residues 274–289 of COX-1 (Morita et al., 1995) was used to determine the level of

COX-1 protein or any truncated product from the disrupted gene that includes these residues. Amino acids 274–289 would still be present in a truncated protein if it were made, as the codons for these amino acids are 5′ to the site of gene disruption. Western blot analysis (Figure 2B) shows that the normal 70 kDa COX-1 protein is readily detectable in kidney, stomach, and colon microsomes of wild-type F2 *Ptgs1* mice. Neither normal-sized COX-1 protein nor any smaller fragment is detected in the same tissues from the homozygous mutant mice. Figure 2C (upper panel) demonstrates that COX-1 protein levels are not significantly affected by lipopolysaccharide (LPS) in macrophages from wild-type mice and, as expected, are not detectable in the homozygous mutant mice. The bottom panel of Figure 2C shows that LPS induces the COX-2 protein about equally in peritoneal macrophages from COX-1 wild-type and homozygous mutant mice. COX-2 message and PGE_2 production are also induced about equally in the macrophages from wild-type and homozygous mutant mice (data not shown). These results indicate that disruption of *Ptgs1* prevents constitutive synthesis of COX-1 but does not alter *Ptgs2* inducibility in macrophages.

PGE_2 Production in Peritoneal Macrophages

To determine the effect of *Ptgs1* gene disruption on prostaglandin biosynthesis, we isolated and analyzed peritoneal macrophages for their basal (not LPS-stimulated) level of PGE_2 production with exogenous AA as the substrate. The data in Figure 3 show that basal PGE_2 production is reduced about 70% in heterozygous mice and is reduced more than 99% in homozygous mutant mice. The basal levels of PGE_2 production in the wild-type and heterozygous mice were not altered by a 6 hr incubation in medium containing dexamethasone, indicating that PGE_2 production is due to COX-1 and not to COX-2, the production of which is inhibited by dexamethasone. These data show that basal PGE_2 production is virtually absent in macrophages from the *Ptgs1* disrupted mice and that COX-2 contributes little to basal levels of PGE_2 from exogenous AA in unstimulated macrophages.

Gastric Ulceration

The COX-1 homozygous mutant mice from F2 and subsequent generations did not have gross or microscopic gastric lesions; therefore, the absence of COX-1 alone is not sufficient to cause lesions. Because COX-1 is a target for NSAIDs (Xie et al., 1992; Seibert et al., 1994; Masferrer et al., 1994; Mitchell et al., 1994; Seibert and Masferrer, 1994) and because NSAID inhibition of COX-1 has been thought causal in the induction of gastrointestinal lesions, we investigated the possibility that COX-1-deficient mice have altered sensitivity to indomethacin, an NSAID known to induce stomach ulceration in mice (Yokoyama et al., 1985; Rainsford, 1987; Ettarh and Carr, 1993). First, a dose response was determined by administering indomethacin to heterozygous F2 mice by gavage at doses ranging from 10 to 80 mg/kg (data not shown). Doses of 10 and 20 mg/kg were found to be in the lower, but still detectable, response range. The data in Figure 4 show that F2 and F3 wild-type and homozygous mutant mice treated with 20 mg/kg have about an equal number of ulcers, although the percent of surface area ulcerated is somewhat reduced in the homozygous mutant mice ($p <$

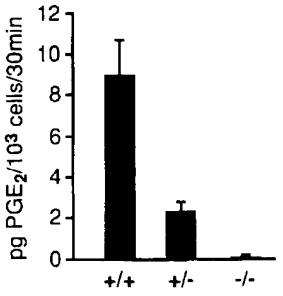

Figure 3. Production of PGE_2 by Peritoneal Macrophages

PGE_2 levels were determined by radioimmunoassay, and the data are expressed as picograms of PGE_2 per 10^3 cells. Data are for macrophages from five mice for each genotype (wild type, heterozygous, and homozygous mutant). Data are presented as the mean ± SEM.

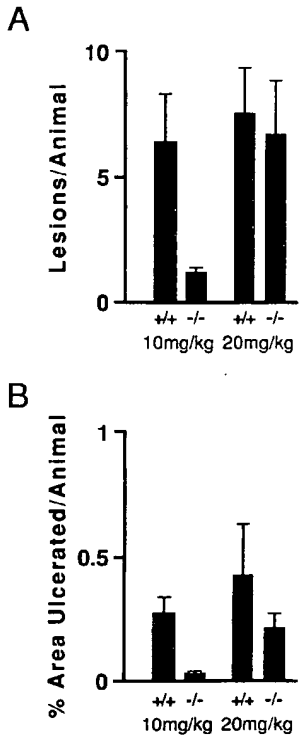

Figure 4. Induction of Stomach Ulceration by Indomethacin in Wild-Type and Homozygous Mutant Mice

(A) Data are expressed as the number of lesions of glandular stomach ulcers per animal at 10 and 20 mg/kg indomethacin. (B) Data are expressed as the percent ulcerated area of the total glandular stomach surface area at 10 and 20 mg/kg indomethacin. For (A) and (B), 10 mg/kg wild type (n = 9), homozygous mutant (n = 6); 20 mg/kg wild-type (n = 7), homozygous mutant (n = 8). Data are presented as the mean ± SEM.

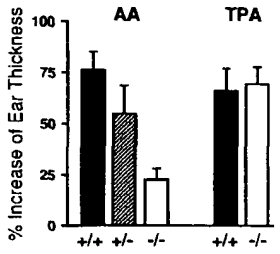

Figure 5. Induction of Ear Inflammation by AA and TPA

Data are expressed as the percent increase over pretreatment ear thickness. For AA and TPA treatments, five and four mice, respectively, of the F2 genotype indicated (wild type, heterozygous, homozygous mutant) were used. The mean control ear thickness was 0.22 ± 0.02 mm. Data are presented as the mean ± SEM.

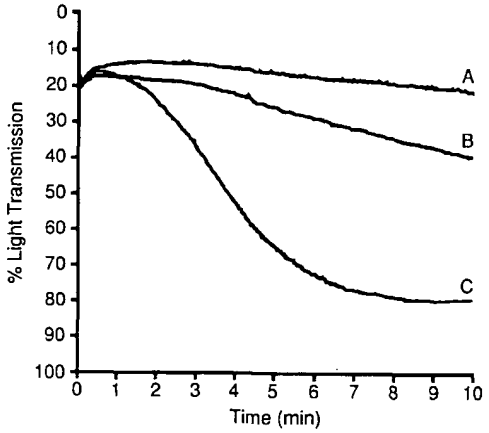

Figure 6. AA-Induced Aggregation of Platelets from Wild-Type F2 and Homozygous F2 Mutant Mice

(A) Solvent control with platelets from wild-type mice. (B) Platelets plus AA from homozygous mutant mice. (C) Platelets plus AA from wild-type mice.

0.36). At the 10 mg/kg dose, the homozygous mutant mice had statistically fewer lesions ($p < 0.04$), and a lower percent of stomach surface area was ulcerated ($p < 0.06$) than in the wild-type mice.

A possible means whereby gastric cytoprotection might be achieved in the COX-1 homozygous mutant mice is via a compensatory production of prostaglandins by the COX-2 isoform. To investigate this possibility, we determined PGE_2 levels in the glandular stomachs of COX-1 homozygous mutant mice and compared these levels with those in wild-type mice, untreated or after gavage with 40 mg/kg of indomethacin. Untreated wild-type levels were 113,430 ± 5,430 pg per milligram of tissue ($n = 2$); treated wild-type were 353 ± 126 pg per milligram of tissue ($n = 2$); homozygous mutant levels were 713 ± 265 pg per milligram of tissue ($n = 2$). Thus, it appears that COX-2 is contributing little to PGE_2 production in the stomach of COX-1 homozygous mutant mice.

Ear Inflammation

A standard ear swelling assay (Gad et al., 1986; Opas et al., 1985) was used to determine whether mice with the *Ptgs1* gene disruption had an altered inflammatory response to chemical challenge. The mice used in these studies were littermates from the F2 generation. Figure 5 shows that when AA was administered topically, the homozygous mutant mice had a significantly reduced ($p < 0.002$) inflammatory response (about 30% normal). The heterozygous mice also had a decreased response, although it did not reach statistical significance ($p < 0.26$). In contrast, inflammation in response to the potent tumor promoter tetradecanoyl phorbol acetate (TPA) did not differ in the wild-type and homozygous mutant mice. By 6 hr after treatment with AA, ear thickness had returned to normal, while TPA-treated ears remained inflamed for at least 18 hr after treatment.

In gene targeting experiments in which breeding is not confined to a single inbred strain of mice, the cosegregation of strain differences in genes linked to the target locus must be considered (Smithies and Maeda, 1995). In the present instance, this complication is effectively eliminated by the observations of Morham et al. (1995); their wild-type F2 *Ptgs2* animals, in which the wild-type *Ptgs1* locus and linked genes from the B6 and 129 strains can occur in all combinations, did not differ significantly in response to AA from our F2 wild-type animals carrying only the *Ptgs1* B6/B6 combination.

Platelet Aggregation

COX-1 is considered the key enzyme for generating prostaglandins involved in platelet aggregation (Funk et al., 1991). We therefore investigated the ability of platelets from wild-type and homozygous mutant mice to aggregate in vitro. The curves in Figure 6 show that platelets from homozygous mutant mice aggregate more slowly and to a lesser extent in response to AA than do platelets from wild-type mice.

Reproductive Capability of Homozygous Mutant Mice

Table 1 lists the frequency of live births and the litter sizes resulting from different heterozygous and homozygous matings. When homozygous mutant females are mated with homozygous mutant males, almost all of the pups are found dead, even though the litter sizes are normal. The cause of pup death has not been determined. In con-

Table 1. Effect of Parental Genotype on Litter Size and Pup Survival

Pairing		Postnatal Survivors	Average
Male	Female	per Total Births[a]	Litter Size[a]
Homozygous × homozygous[b]		4 of 39	7.8 ± 1.2
Heterozygous × homozygous		20 of 26	6.5 ± 1.0
Homozygous × heterozygous		27 of 31	7.8 ± 0.5

[a] Data are based on four litters for the heterozygous × homozygous and five litters for the homozygous × homozygous pairs mated.
[b] Four different homozygous females, one of which produced two litters.

trast, when homozygous mutant females are mated with heterozygous males, the number of surviving pups and the litter sizes are close to normal, with the number of pups having the heterozygous and homozygous mutant genotypes being essentially equal. The breeding of homozygous mutant males to heterozygous females likewise results in normal pup survival and litter size. These data indicate that both homozygous mutant males and homozygous mutant females are fertile, but that pup survival is decreased when homozygous mutant mice are mated to each other; in this situation, neither the female nor the pups have functional COX-1.

Discussion

In the present study, we have used homologous recombination to disrupt the mouse *Ptgs1* gene that encodes COX-1. The major findings are that COX-1-deficient mice are generally healthy, do not have spontaneous stomach ulcers, and show less gastric ulceration than wild-type mice after gavage with indomethacin. The homozygous mutant mice also have a reduced inflammatory response to AA, and homozygous mutant × homozygous mutant matings result in reduced pup survival.

Because the stomach and kidney are two tissues where COX activity had been thought essential for proper function (Robert, 1975, 1979; Clive and Stoff, 1984; Black, 1986; Brooks and Day, 1991; Price and Fletcher, 1990; Simon, 1994), the lack of pathology in these tissues of COX-1-deficient mice was surprising. Furthermore, based on the hypotheses that COX-1 is a housekeeping enzyme and that NSAID inhibition of COX-1 is responsible for stomach ulceration, we had expected that COX-1 deficiency might lead to spontaneous stomach ulceration or bleeding in the homozygous mutant mouse. But the gastrointestinal tissues of the homozygous mutants were not distinguishable from wild type. Thus, absence of COX-1 is not sufficient to cause stomach ulceration in mice. Furthermore, measurement of PGE_2 in the glandular stomach of COX-1 homozygous mutant mice indicates that PGE_2 levels per milligram of tissue are less than 1% of the levels observed in wild-type mice. This reduction is consistent with that observed in peritoneal macrophages from homozygous mutant mice (Figure 3) and is approximately equal to the levels observed in the stomachs of wild-type mice treated with 40 mg/kg indomethacin. The low level of PGE_2 in the glandular stomach of the homozygous mutant mice coupled with the finding that COX-2 is undetectable by Western blot analysis (data not shown) suggests that compensation by COX-2 is not a significant factor in this tissue in COX-1 homozygous mutant mice.

Because of the absence of spontaneous gastric ulceration in the COX-1-deficient mice, we determined whether they have an altered sensitivity to NSAID-induced gastric ulceration. For this we chose indomethacin, which is widely used in NSAID studies of gastric ulceration and is known to induce ulceration in the mouse stomach (Yokoyama et al., 1985; Rainsford, 1987; Ettarh and Carr, 1993). Our experiments confirm that wild-type mice are sensitive to stomach ulceration by indomethacin gavaged at either 10 or 20 mg/kg, doses commonly used to cause ulceration in rats (Futaki et al., 1993; Rainsford, 1993; Beck et al., 1990). Surprisingly, we found that the homozygous *Ptgs1* homozygous mutants are less sensitive to indomethacin-induced stomach ulceration than are the wild-type mice: the absence of COX-1 decreases rather than increases the incidence of ulceration after indomethacin treatment. Thus, these observations suggest that indomethacin-induced gastric ulceration may be due to mechanisms other than (or in addition to) COX-1 inhibition. Alternatively, in the development of gastric ulcers, lack of COX-1 activity due to gene disruption may not be equivalent to the inhibition of COX-1 activity by indomethacin. As described by Morham et al. (1995), mice deficient in COX-2 also show no spontaneous stomach ulcers or overt intestinal lesions. These data emphasize that the relationship between inhibition of COX activity and ulceration is complex, and they illustrate that the COX-1- and COX-2-deficient mice provide novel ways of studying isoform-specific NSAIDs and for identifying mechanisms in addition to COX inhibition that may be involved in the ulcerative process.

The kidneys of the COX-1-deficient mice showed only minimal abnormalities even at 5 months of age. Therefore, absence of COX-1 in the kidney is not deleterious under normal physiological conditions.

Prostaglandins have key functions in various stages of the reproductive process, ranging from ovulation and spermatogenesis to parturition (Thorburn, 1991, 1992; Zahradnik et al., 1992). Which COX isoform is involved in each of these stages is not known, except for ovulation, when it appears that COX-2 is the important form (Sirois et al., 1992). Our studies show that neither male nor famale fertility appears to be affected by lack of COX-1 (Table 1), but they clearly show that complete lack of COX-1, such as occurs in homozygous mutant × homozygous mutant matings, severely impedes survival of pups perinatally. Because prostaglandins are known to be involved in the initiation of labor (Kelly, 1994), it may be the onset of labor that is impaired. However, the normal litter size and pup survival seen when heterozygous males are mated with homozygous mutant females show that absence of COX-1 synthesis in the mother can be overcome by the presence of COX-1 in as few as 50% of the pups or in their placental material. From these matings, heterozygous and homozygous mutant pups are born in about equal numbers. The mating of homozygous mutant males with heterozygous females likewise results in normal litter sizes and pup survival. The most likely explanation for these observations is that prostaglandins from the COX-1 pathway in either the maternal or fetal tissues are essential for normal parturition. Further studies are needed to elucidate the roles of the COX isoforms in pregnancy and parturition, and the COX-deficient mice should provide useful models for such studies.

The decrease in AA-induced platelet aggregation seen in our COX-1-deficient mice accords well with existing data that platelets contain the constitutively produced isoform

COX-1 (Funk et al., 1991). Since platelets lack nuclei, this deficiency cannot be overcome by any compensatory induction of *Ptgs2* transcription followed by COX-2 synthesis.

The COX-1-deficient mice clearly have a reduction in their responses to AA, although their responses to TPA do not differ from wild type. However, the COX-2-deficient animals are as sensitive to inflammation caused by both AA and TPA as are wild-type mice. A possible reason for the differences between AA and TPA effects in the COX-1-deficient mice is that AA in wild-type mice (and COX-2-deficient mice) can be immediately metabolized by the constitutive COX-1 enzyme to PGH_2 and subsequently to PGE_2, which contributes to edema and inflammation. Since this cannot occur in the COX-1 homozygous mutant mice, less inflammation occurs. TPA, on the other hand, does not interact directly with COX-1 but is known to induce the synthesis of other enzymes, including COX-2 in vitro (Kujubu et al., 1991; DuBois et al., 1994a) and in vivo (Muller-Decker et al., 1995). The finding that TPA-induced inflammation is equal in COX-2 homozygous mutant mice and wild-type mice indicates that COX-2 is not essential for this type of inflammation to occur in the skin of these mice. Thus, it appears that COX-1 can contribute to inflammation. More detailed studies of the responses of the COX-1- or COX-2-deficient mice will be needed to indicate the relative roles of the two isoforms following different types of inflammatory stimuli.

The major conclusions from the present study are that lack of COX-1 does not cause spontaneous gastric ulceration and decreases indomethacin-induced ulceration, that it decreases inflammatory responses to AA, that it decreases platelet aggregation, but that it has no other overt systemic effects except those associated with parturition. Lack of COX-2, as reported by Morham et al. (1995), also does not cause spontaneous stomach ulceration, but, in contrast with lack of COX-1, it has no effect on inflammatory responses to AA, causes severe kidney disease, and leads to spontaneous peritonitis in some animals.

Experimental Procedures

Targeting Vector Construction

A Charon 35 genomic library containing DNA from E14TG2a mouse ES cells (Hooper et al., 1987) was screened with a 357 bp probe (see below) for the 5′ end of exon 11 of the mouse *Ptgs1* gene. A clone containing approximately 15 kb of the 3′ end of the *Ptgs1* gene was isolated, and overlapping 6 kb XbaI and ClaI fragments (Figure 1A) were subcloned into Bluescript. A 4.3 kb NotI–XhoI fragment of the XbaI subclone was inserted into the pPNT vector (Tybulewicz et al., 1991) 5′ to the *Neo* gene, and a 2.3 kb BamHI fragment from the ClaI subclone was inserted 3′ to the *Neo* gene to produce the targeting construct (Figure 1B).

Cell Culture and Targeting

E14TG2a ES cells were cultured by conventional methods on feeder cells. The targeting vector was linearized with NotI and electroporated at 5 nM into about 10^8 trypsinized ES cells using a 1 s pulse at 300 V and 200 μF. Positive/negative selection was performed with G418 and ganciclovir (Mansour et al., 1988) with ganciclovir providing about a 10-fold enrichment over G418 alone. Ganciclovir- and G418-resistant colonies were isolated 8–9 days after electroporation and transferred individually to 24-well plates previously seeded with feeder cells. The cells were trypsinized 2 days later and reseeded into 6-well plates. After 2 days the cells were trypsinized, and about half were frozen at −80°C. DNA isolated from the remaining half was used for PCR and genomic Southern blot analysis.

PCR and Southern Blot Analysis

PCR was the initial screen for identifying targeted ES cells and mice carrying the disrupted *COX-1* gene (Kim and Smithies, 1988). A primer specific for the *Neo* gene and a second primer specific for a genomic sequence 3′ to sequences in the targeting construct (Figure 1C) produced a single 2.4 kb band diagnostic of targeting. Genomic Southern blots were produced by standard techniques and probed with a random primer ^{32}P-labeled probe (Stratagene Prime-It II) made from the 357 bp PCR fragment specific for the 5′ region of exon 11. DNA was isolated from mouse tails to determine genotype.

Northern Blot Analysis

Total RNA was isolated from tissues by homogenizing them in TRIzol (Life Technologies) or from cells by scraping directly into TRIzol as recommended by the supplier. Each sample (15 μg) was electrophoresed in a 2.2 M formaldehyde–0.9% agarose gel. After capillary transfer, the blot was hybridized with a random primer ^{32}P-labeled probe made from the exon 11–specific 357 bp PCR fragment or a 1.7 kb *COX-1* cDNA fragment (Oxford Biomedical Research Corporation). Blots were stripped and probed for actin to ensure equal loading of RNA.

Western Blot Analysis

To prepare microsomes, we homogenized tissues in buffer (0.1 M Tris–HCl [pH 7.4], 2 mM EDTA, 10 mg/ml leupeptin, 20 mg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride) and then sonicated them at 30% power (Fisher Scientific, Model F50) three times for 15 s. The homogenates were centrifuged at 10,000 × g for 15 min at 4°C. The resulting supernatants were centrifuged at 100,000 × g for 1 hr at 4°C, and the microsomal pellets were sheared in buffer (100 mM Tris [pH 6.8], 8% SDS, 20% glycerol) with a 25-gauge needle. An aliquot was removed for protein determination (Bio-Rad DC) before boiling with bromophenol blue (0.05% [w/v]) and 2-mercaptoethanol (6% [v/v]).

For immunoblot analysis, 40 μg of microsomal protein (kidney and stomach), 20 μg of microsomal protein (colon), or 10 μg of protein from cell lysate (macrophages) were separated by SDS–PAGE using the Mini-PROTEAN II electrophoretic apparatus (Bio-Rad). Proteins were transferred onto Hybond–ECL nitrocellulose (Amersham) using the Mini Trans-Blot electrophoretic transfer cell system (Bio-Rad). Membranes were blocked in 5% nonfat milk–Tris-buffered saline with 0.1% Tween 20 (TBST) before incubating with a rabbit antibody to murine COX-1 provided by Dr. D. DeWitt (Morita et al., 1995) or to COX-2 (Cayman Chemical). Blots were incubated with anti-rabbit IgG horseradish peroxidase–linked secondary antibody (Boehringer Mannheim) in TBST and 1% nonfat milk. Chemiluminescent detection was performed using reagents from Amersham, and bands were visualized after exposure to Hyperfilm–ECL (Amersham).

Indomethacin-Induced Stomach Ulceration

Indomethacin was suspended in 1% methyl cellulose at the concentration of 1 or 2 mg/ml and given at the stated doses by gavage. All animals were fasted 16–18 hr prior to gavage. Animals were euthanized using CO_2 6 hr after treatment, and their stomaches were removed and opened along the lesser curvature. Stomach lesions were scored as described by Ghanayem et al. (1987). The number of lesions were counted, an enlarged image of the formalin-fixed glandular stomach and of each individual lesion was traced, and the area of each lesion was determined using a computer-assisted image analysis system. The total area of the stomach was traced and measured. The area of all lesions in each stomach was calculated and divided by the area of the glandular stomach to derive the percent of area with lesions. All samples were scored blind. No ulceration was shown by eight wild-type mice that received vehicle alone.

Mouse Ear Inflammation Assay

AA (2 mg per 10 μl) or TPA (1 μg per 10 μl) in acetone was applied

to the inside of the left ear and 10 μl of acetone was applied to the right ear as described by Opas et al. (1985). Ear swelling was determined after 2 hr for AA or 6 hr for TPA by the method of Gad et al. (1986).

Macrophage Isolation and PGE_2 Analysis

Peritoneal macrophages were isolated and LPS stimulated by modification of the procedure of Watanabe et al. (1994). Thioglycolate-elicited macrophages were isolated by peritoneal lavage with 5 ml of cold RPMI 1640 medium. Macrophages were seeded at 1×10^7 to 2.5×10^7 cells per 60 mm dish, depending on yield, and allowed to attach for 2 hr in a humidified incubator with 5% CO_2 in air. The plates were then washed with Hank's balanced salt solution to remove nonadhering cells, and medium containing 1% serum with or without LPS (10 μg/ml) was then added. Attached cell numbers were determined by counting with an eyepiece micrometer. After a 6 hr incubation, the medium was removed and replaced for 30 min with medium containing 10 μM AA. Subsequent analysis for PGE_2 in the medium was by a competitive radioimmunoassay (Amersham).

Platelet Aggregation

Platelet aggregation was carried out as described by Paigen et al. (1987) using AA to induce aggregation. For each assay, blood was pooled from two mice (about 1 ml in total volume) and centrifuged to prepare platelet-rich and then platelet-poor plasma. The assay was conducted with 300 μl of plasma at 3×10^8 platelets per milliliter. Aggregation was induced by adding 15 μl of Na–AA (22 mg/ml in Na_2CO_3 buffer). Turbidity was measured with a Lumi-Aggregometer.

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Recommended Concentrations and Doses of Mepivacaine Hydrochloride

Procedure	Concentration	Total Dose mL	Total Dose mg	Comments
Cervical, brachial, intercostal, pudendal nerve block	1%	5–40	50–400	Pudendal block: one half of total dose injected each side.
	2%	5–20	100–400	
Transvaginal block (paracervical plus pudendal)	1%	up to 30 (both sides)	up to 300 (both sides)	One half of total dose injected each side. See PRECAUTIONS.
Paracervical block	1%	up to 20 (both sides)	up to 200 (both sides)	One half of total dose injected each side. This is maximum recommended dose per 90-minute period in obstetrical and non-obstetrical patients. Inject slowly, 5 minutes between sides. See PRECAUTIONS.
Caudal and Epidural block	1%	15–30	150–300	*Use only single-dose vials which do not contain a preservative.
	1.5%	10–25	150–375	
	2%	10–20	200–400	
Infiltration	1%	up to 40	up to 400	An equivalent amount of a 0.5% solution (prepared by diluting the 1% solution with Sodium Chloride Injection, USP) may be used for large areas.
Therapeutic block (pain management)	1%	1–5	10–50	
	2%	1–5	20–100	

Unused portions of solutions not containing preservatives should be discarded.

*Dosage forms listed as POLOCAINE-MPF (Mepivacaine HCl Injection, USP) are single-dose solutions which do not contain a preservative.

their use. Immediately after the institution of these ventilatory measures, the adequacy of the circulation should be evaluated. Supportive treatment of circulatory depression may require administration of intravenous fluids, and when appropriate, a vasopressor dictated by the clinical situation such as ephedrine or epinephrine to enhance myocardial contractile force).

Endotracheal intubation, employing drugs and techniques familiar to the clinician may be indicated after initial administration of oxygen by mask, if difficulty is encountered in the maintenance of patent airway or if prolonged ventilatory support (assisted or controlled) is indicated.

Recent clinical data from patients experiencing local anesthetic induced convulsions demonstrated rapid development of hypoxia, hypercarbia, and acidosis within a minute of the onset of convulsions. These observations suggest that oxygen consumption and carbon dioxide production are greatly increased during local anesthetic convulsions and emphasize the importance of immediate and effective ventilation with oxygen which may avoid cardiac arrest.

If not treated immediately, convulsions with simultaneous hypoxia, hypercarbia, and acidosis, plus myocardial depression from the direct effects of the local anesthetic may result in cardiac arrhythmias, bradycardia, asystole, ventricular fibrillation, or cardiac arrest. Respiratory abnormalities, including apnea, may occur. Underventilation or apnea due to unintentional subarachnoid injection of local anesthetic solution may produce these same signs and also lead to cardiac arrest if ventilatory support is not instituted. If cardiac arrest should occur, standard cardiopulmonary resuscitative measures should be instituted and maintained for a prolonged period if necessary. Recovery has been reported after prolonged resuscitative efforts.

The supine position is dangerous in pregnant women at term because of aortocaval compression by the gravid uterus. Therefore, during treatment of systemic toxicity, maternal hypotension, or fetal bradycardia following regional block, the parturient should be maintained in the left lateral decubitus position if possible, or manual displacement of the uterus off the great vessels should be accomplished.

The mean seizure dosage of mepivacaine in rhesus monkeys was found to be 18.8 mg/kg with mean arterial plasma concentration of 24.4 μg/mL. The intravenous and subcutaneous LD_{50} in mice is 23 mg/kg to 35 mg/kg and 280 mg/kg respectively.

DOSAGE AND ADMINISTRATION

The dose of any local anesthetic administered varies with the anesthetic procedure, the area to be anesthetized, the vascularity of the tissues, the number of neuronal segments to be blocked, the depth of anesthesia and degree of muscle relaxation required, the duration of anesthesia desired, individual tolerance and the physical condition of the patient. The smallest dose and concentration required to produce the desired result should be administered. Dosages of mepivacaine hydrochloride should be reduced for elderly and debilitated patients and patients with cardiac and/or liver disease. The rapid injection of a large volume of local anesthetic solution should be avoided and fractional doses should be used when feasible.

For specific techniques and procedures, refer to standard textbooks.

The recommended single ***adult*** dose (or the total of a series of doses given in one procedure) of mepivacaine hydrochloride for unsedated, healthy, normal-sized individuals should not usually exceed 400 mg. The recommended dosage is based on requirements for the average adult and should be reduced for elderly or debilitated patients.

While maximum doses of 7 mg/kg (550 mg) have been administered without adverse effect, these are not recommended, except in exceptional circumstances and under no circumstances should the administration be repeated at intervals of less than 1½ hours. The total dose for any 24-hour period should not exceed 1,000 mg because of a slow accumulation of the anesthetic or its derivatives or slower than normal metabolic degradation or detoxification with repeat administration. (See CLINICAL PHARMACOLOGY and PRECAUTIONS.)

Pediatric patients tolerate the local anesthetic as well as adults. However, the pediatric dose should be ***carefully measured*** as a percentage of the total adult dose ***based on weight***, and should not exceed 5 mg/kg to 6 mg/kg (2.5 mg/lb to 3 mg/lb) in pediatric patients, especially those weighing less than 30 lbs. In pediatric patients ***under 3 years of age or weighing less than 30 lbs*** concentrations less than 2% (eg, 0.5% to 1.5%) should be employed.

Unused portions of solutions not containing preservatives, ie, those supplied in single-dose vials, should be discarded following initial use.

This product should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Solutions which are discolored or which contain particulate matter should not be administered.

[See table above]

HOW SUPPLIED

Single-dose vials and multiple-dose vials of POLOCAINE may be sterilized by autoclaving at 15 pound pressure, 121°C (250°F) for 15 minutes. Solutions of POLOCAINE may be reautoclaved when necessary. Do not administer solutions which are discolored or which contain particulate matter.

THESE SOLUTIONS ARE NOT INTENDED FOR SPINAL ANESTHESIA OR DENTAL USE.

POLOCAINE-MPF (Mepivacaine HCl Injection, USP) without preservatives is available as follows:

1% Single-dose vials of 30 mL (NDC 0186-0412-01)
1.5% Single-dose vials of 30 mL (NDC 0186-0418-01)
2% Single-dose vials of 20 mL (NDC 0186-0422-01)

POLOCAINE (Mepivacaine HCl Injection, USP) with preservatives is available as follows:

1% Multiple-dose vials of 50 mL (NDC 0186-0410-01)
2% Multiple-dose vials of 50 mL (NDC 0186-0420-01)

Store at controlled room temperature 15–30°C (59–86°F); brief exposure up to 40°C (104°F) does not adversely affect the product.

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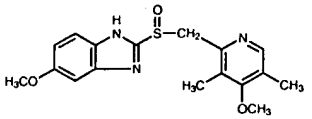
721668-03
Rev. 01/02

PRILOSEC® Rx
[prī-lō-sĕk]
(OMEPRAZOLE)
DELAYED-RELEASE CAPSULES

DESCRIPTION

The active ingredient in PRILOSEC (omeprazole) Delayed-Release Capsules is a substituted benzimidazole, 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1*H*-benzimidazole, a compound that inhibits gastric acid secretion. Its empirical formula is $C_{17}H_{19}N_3O_3S$, with a molecular weight of 345.42. The structural formula is:



Omeprazole is a white to off-white crystalline powder which melts with decomposition at about 155°C. It is a weak base, freely soluble in ethanol and methanol, and slightly soluble in acetone and isopropanol and very slightly soluble in water. The stability of omeprazole is a function of pH; it is rapidly degraded in acid media, but has acceptable stability under alkaline conditions.

PRILOSEC is supplied as delayed-release capsules for oral administration. Each delayed-release capsule contains either 10 mg, 20 mg or 40 mg of omeprazole in the form of enteric-coated granules with the following inactive ingredients: cellulose, disodium hydrogen phosphate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, mannitol, sodium lauryl sulfate and other ingredients. The capsule shells have the following inactive ingredients: gelatin-NF, FD&C Blue #1, FD&C Red #40, D&C Red #28, titanium dioxide, synthetic black iron oxide, isopropanol, butyl alcohol, FD&C Blue #2, D&C Red #7 Calcium Lake, and, in addition, the 10 mg and 40 mg capsule shells also contain D&C Yellow #10.

CLINICAL PHARMACOLOGY

Pharmacokinetics and Metabolism: Omeprazole

PRILOSEC Delayed-Release Capsules contain an enteric-coated granule formulation of omeprazole (because omeprazole is acid-labile), so that absorption of omeprazole begins only after the granules leave the stomach. Absorption is rapid, with peak plasma levels of omeprazole occurring within 0.5 to 3.5 hours. Peak plasma concentrations of omeprazole and AUC are approximately proportional to doses up to 40 mg, but because of a saturable first-pass effect, a greater than linear response in peak plasma concentration and AUC occurs with doses greater than 40 mg. Absolute bioavailability (compared to intravenous administration) is about 30–40% at doses of 20–40 mg, due in large part to presystemic metabolism. In healthy subjects the plasma half-life is 0.5 to 1 hour, and the total body clearance is 500–600 mL/min. Protein binding is approximately 95%. The bioavailability of omeprazole increases slightly upon repeated administration of PRILOSEC Delayed-Release Capsules.

Following single dose oral administration of a buffered solution of omeprazole, little if any unchanged drug was excreted in urine. The majority of the dose (about 77%) was eliminated in urine as at least six metabolites. Two were identified as hydroxyomeprazole and the corresponding carboxylic acid. The remainder of the dose was recoverable in feces. This implies a significant biliary excretion of the metabolites of omeprazole. Three metabolites have been identified in plasma — the sulfide and sulfone derivatives of omeprazole, and hydroxyomeprazole. These metabolites have very little or no antisecretory activity.

In patients with chronic hepatic disease, the bioavailability increased to approximately 100% compared to an I.V. dose, reflecting decreased first-pass effect, and the plasma half-life of the drug increased to nearly 3 hours compared to the half-life in normals of 0.5–1 hour. Plasma clearance averaged 70 mL/min, compared to a value of 500–600 mL/min in normal subjects.

In patients with chronic renal impairment, whose creatinine clearance ranged between 10 and 62 mL/min/1.73 m^2, the disposition of omeprazole was very similar to that in healthy volunteers, although there was a slight increase in bioavailability. Because urinary excretion is a primary route of excretion of omeprazole metabolites, their elimination slowed in proportion to the decreased creatinine clearance. The elimination rate of omeprazole was somewhat decreased in the elderly, and bioavailability was increased. Omeprazole was 76% bioavailable when a single 40 mg oral dose of omeprazole (buffered solution) was administered to healthy elderly volunteers, versus 58% in young volunteers given the same dose. Nearly 70% of the dose was recovered

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Prilosec—Cont.

in urine as metabolites of omeprazole and no unchanged drug was detected. The plasma clearance of omeprazole was 250 mL/min (about half that of young volunteers) and its plasma half-life averaged one hour, about twice that of young healthy volunteers.

In pharmacokinetic studies of single 20 mg omeprazole doses, an increase in AUC of approximately four-fold was noted in Asian subjects compared to Caucasians.

Dose adjustment, particularly where maintenance of healing of erosive esophagitis is indicated, for the hepatically impaired and Asian subjects should be considered.

PRILOSEC Delayed-Release Capsule 40 mg was bioequivalent when administered with and without applesauce. However, PRILOSEC Delayed-Release Capsule 20 mg was not bioequivalent when administered with and without applesauce. When administered with applesauce, a mean 25% reduction in C_{max} was observed without a significant change in AUC for PRILOSEC Delayed-Release Capsule 20 mg. The clinical relevance of this finding is unknown.

The pharmacokinetics of omeprazole have been investigated in pediatric patients of different ages.

Pharmacokinetic Parameters of Omeprazole Following Single and Repeated Oral Administration in Pediatric Populations Compared to Adults

Single or Repeated Oral Dosing/ Parameter	Children† < 20 kg 2–5 years 10 mg	Children† > 20 kg 6–16 years 20 mg	Adults‡ (mean 76 kg) 23–29 years (n=12)
Single Dosing			
C_{max}* (ng/mL)	288 (n=10)	495 (n=49)	668
AUC* (ng h/mL)	511 (n=7)	1140 (n=32)	1220
Repeated Dosing			
C_{max}* (ng/mL)	539 (n=4)	851 (n=32)	1458
AUC* (ng h/mL)	1179 (n=2)	2276 (n=23)	3352

Note: * = plasma concentration adjusted to an oral dose of 1 mg/kg.
†Data from single and repeated dose studies
‡Data from a single and repeated dose study
Doses of 10, 20 and 40 mg Omeprazole as Enteric-Coated Granules

Following comparable mg/kg doses of omeprazole, younger children (2–5 years) have lower AUCs than children 6–16 years or adults; AUCs of the latter two groups did not differ, (see DOSAGE AND ADMINISTRATION, Pediatric Patients).

Pharmacokinetics: Combination Therapy with Antimicrobials

Omeprazole 40 mg daily was given in combination with clarithromycin 500 mg every 8 hours to healthy adult male subjects. The steady state plasma concentrations of omeprazole were increased (C_{max}, AUC_{0-24}, and $T_{1/2}$ increases of 30%, 89% and 34% respectively) by the concomitant administration of clarithromycin. The observed increases in omeprazole plasma concentration were associated with the following pharmacological effects. The mean 24-hour gastric pH value was 5.2 when omeprazole was administered alone and 5.7 when co-administered with clarithromycin.

The plasma levels of clarithromycin and 14-hydroxy-clarithromycin were increased by the concomitant administration of omeprazole. For clarithromycin, the mean C_{max} was 10% greater, the mean C_{min} was 27% greater, and the mean AUC_{0-8} was 15% greater when clarithromycin was administered with omeprazole than when clarithromycin was administered alone. Similar results were seen for 14-hydroxy-clarithromycin, the mean C_{max} was 45% greater, the mean C_{min} was 57% greater, and the mean AUC_{0-8} was 45% greater. Clarithromycin concentrations in the gastric tissue and mucus were also increased by concomitant administration of omeprazole.

Clarithromycin Tissue Concentrations 2 hours after Dose[1]

Tissue	Clarithromycin	Clarithromycin + Omeprazole
Antrum	10.48 ± 2.01 (n = 5)	19.96 ± 4.71 (n = 5)
Fundus	20.81 ± 7.64 (n = 5)	24.25 ± 6.37 (n = 5)
Mucus	4.15 ± 7.74 (n = 4)	39.29 ± 32.79 (n = 4)

[1]Mean ± SD (μg/g)

For information on clarithromycin pharmacokinetics and microbiology, consult the clarithromycin package insert, CLINICAL PHARMACOLOGY section.

The pharmacokinetics of omeprazole, clarithromycin, and amoxicillin have not been adequately studied when all three drugs are administered concomitantly.

For information on amoxicillin pharmacokinetics and microbiology, see the amoxicillin package insert, ACTIONS, PHARMACOLOGY and MICROBIOLOGY sections.

Pharmacodynamics

Mechanism of Action

Omeprazole belongs to a new class of antisecretory compounds, the substituted benzimidazoles, that do not exhibit anticholinergic or H_2 histamine antagonistic properties, but that suppress gastric acid secretion by specific inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell. Because this enzyme system is regarded as the acid (proton) pump within the gastric mucosa, omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. This effect is dose-related and leads to inhibition of both basal and stimulated acid secretion irrespective of the stimulus. Animal studies indicate that after rapid disappearance from plasma, omeprazole can be found within the gastric mucosa for a day or more.

Antisecretory Activity

After oral administration, the onset of the antisecretory effect of omeprazole occurs within one hour, with the maximum effect occurring within two hours. Inhibition of secretion is about 50% of maximum at 24 hours and the duration of inhibition lasts up to 72 hours. The antisecretory effect thus lasts far longer than would be expected from the very short (less than one hour) plasma half-life, apparently due to prolonged binding to the parietal H^+/K^+ ATPase enzyme. When the drug is discontinued, secretory activity returns gradually, over 3 to 5 days. The inhibitory effect of omeprazole on acid secretion increases with repeated once-daily dosing, reaching a plateau after four days.

Results from numerous studies of the antisecretory effect of multiple doses of 20 mg and 40 mg of omeprazole in normal volunteers and patients are shown below. The "max" value represents determinations at a time of maximum effect (2–6 hours after dosing), while "min" values are those 24 hours after the last dose of omeprazole.

Range of Mean Values from Multiple Studies of the Mean Antisecretory Effects of Omeprazole After Multiple Daily Dosing

Parameter	Omeprazole 20 mg Max	Omeprazole 20 mg Min	Omeprazole 40 mg Max	Omeprazole 40 mg Min
% Decrease in Basal Acid Output	78*	58–80	94*	80–93
% Decrease in Peak Acid Output	79*	50–59	88*	62–68
% Decrease in 24-hr. Intragastric Acidity		80–97		92–94

*Single Studies

Single daily oral doses of omeprazole ranging from a dose of 10 mg to 40 mg have produced 100% inhibition of 24-hour intragastric acidity in some patients.

Enterochromaffin-like (ECL) Cell Effects

In 24-month carcinogenicity studies in rats, a dose-related significant increase in gastric carcinoid tumors and ECL cell hyperplasia was observed in both male and female animals (see PRECAUTIONS, Carcinogenesis, Mutagenesis, Impairment of Fertility). Carcinoid tumors have also been observed in rats subjected to fundectomy or long-term treatment with other proton pump inhibitors or high doses of H_2-receptor antagonists.

Human gastric biopsy specimens have been obtained from more than 3000 patients treated with omeprazole in long-term clinical trials. The incidence of ECL cell hyperplasia in these studies increased with time; however, no case of ECL cell carcinoids, dysplasia, or neoplasia has been found in these patients (see CLINICAL PHARMACOLOGY, Pathological Hypersecretory Conditions). However, these studies are of insufficient duration and size to rule out the possible influence of long-term administration of omeprazole on the development of any premalignant or malignant conditions.

Serum Gastrin Effects

In studies involving more than 200 patients, serum gastrin levels increased during the first 1 to 2 weeks of once-daily administration of therapeutic doses of omeprazole in parallel with inhibition of acid secretion. No further increase in serum gastrin occurred with continued treatment. In comparison with histamine H_2-receptor antagonists, the median increases produced by 20 mg doses of omeprazole were higher (1.3 to 3.6 fold vs. 1.1 to 1.8 fold increase). Gastrin values returned to pretreatment levels, usually within 1 to 2 weeks after discontinuation of therapy.

Other Effects

Systemic effects of omeprazole in the CNS, cardiovascular and respiratory systems have not been found to date. Omeprazole, given in oral doses of 30 or 40 mg for 2 to 4 weeks, had no effect on thyroid function, carbohydrate metabolism, or circulating levels of parathyroid hormone, cortisol, estradiol, testosterone, prolactin, cholecystokinin or secretin.

No effect on gastric emptying of the solid and liquid components of a test meal was demonstrated after a single dose of omeprazole 90 mg. In healthy subjects, a single I.V. dose of omeprazole (0.35 mg/kg) had no effect on intrinsic factor secretion. No systematic dose-dependent effect has been observed on basal or stimulated pepsin output in humans. However, when intragastric pH is maintained at 4.0 or above, basal pepsin output is low, and pepsin activity is decreased.

As do other agents that elevate intragastric pH, omeprazole administered for 14 days in healthy subjects produced a significant increase in the intragastric concentrations of viable bacteria. The pattern of the bacterial species was unchanged from that commonly found in saliva. All changes resolved within three days of stopping treatment.

The course of Barrett's esophagus in 106 patients was evaluated in a U.S. double-blind controlled study of PRILOSEC 40 mg b.i.d. for 12 months followed by 20 mg b.i.d. for 12 months or ranitidine 300 mg b.i.d. for 24 months. No clinically significant impact on Barrett's mucosa by antisecretory therapy was observed. Although neosquamous epithelium developed during antisecretory therapy, complete elimination of Barrett's mucosa was not achieved. No significant difference was observed between treatment groups in development of dysplasia in Barrett's mucosa and no patient developed esophageal carcinoma during treatment. No significant differences between treatment groups were observed in development of ECL cell hyperplasia, corpus atrophic gastritis, corpus intestinal metaplasia, or colon polyps exceeding 3 mm in diameter (see CLINICAL PHARMACOLOGY, Enterochromaffin-like (ECL) Cell Effects).

Clinical Studies

Duodenal Ulcer Disease

Active Duodenal Ulcer—In a multi-center, double-blind, placebo-controlled study of 147 patients with endoscopically documented duodenal ulcer, the percentage of patients healed (per protocol) at 2 and 4 weeks was significantly higher with PRILOSEC 20 mg once a day than with placebo ($p \leq 0.01$).

Treatment of Active Duodenal Ulcer % of Patients Healed

	PRILOSEC 20 mg a.m. (n = 99)	Placebo a.m. (n = 48)
Week 2	*41	13
Week 4	*75	27

*($p \leq 0.01$)

Complete daytime and nighttime pain relief occurred significantly faster ($p \leq 0.01$) in patients treated with PRILOSEC 20 mg than in patients treated with placebo. At the end of the study, significantly more patients who had received PRILOSEC had complete relief of daytime pain ($p \leq 0.05$) and nighttime pain ($p \leq 0.01$).

In a multicenter, double-blind study of 293 patients with endoscopically documented duodenal ulcer, the percentage of patients healed (per protocol) at 4 weeks was significantly higher with PRILOSEC 20 mg once a day than with ranitidine 150 mg b.i.d. ($p < 0.01$).

Treatment of Active Duodenal Ulcer % of Patients Healed

	PRILOSEC 20 mg a.m. (n = 145)	Ranitidine 150 mg b.i.d. (n = 148)
Week 2	42	34
Week 4	*82	63

*($p < 0.01$)

Healing occurred significantly faster in patients treated with PRILOSEC than in those treated with ranitidine 150 mg b.i.d. ($p < 0.01$).

In a foreign multinational randomized, double-blind study of 105 patients with endoscopically documented duodenal ulcer, 20 mg and 40 mg of PRILOSEC were compared to 150 mg b.i.d. of ranitidine at 2, 4 and 8 weeks. At 2 and 4 weeks both doses of PRILOSEC were statistically superior (per protocol) to ranitidine, but 40 mg was not superior to 20 mg of PRILOSEC, and at 8 weeks there was no significant difference between any of the active drugs.

Treatment of Active Duodenal Ulcer % of Patients Healed

	PRILOSEC 20 mg (n = 34)	PRILOSEC 40 mg (n = 36)	Ranitidine 150 mg b.i.d. (n = 35)
Week 2	* 83	* 83	53
Week 4	* 97	*100	82
Week 8	100	100	94

*($p \leq 0.01$)

H. pylori Eradication in Patients with Duodenal Ulcer Disease Triple Therapy (PRILOSEC/clarithromycin/amoxicillin)—Three U.S., randomized, double-blind clinical studies in patients with *H. pylori* infection and duodenal ulcer disease (n = 558) compared PRILOSEC plus clarithromycin plus amoxicillin to clarithromycin plus amoxicillin. Two studies (126 and 127) were conducted in patients with an active duodenal ulcer, and the other study (M96-446) was conducted in patients with a history of a duodenal ulcer in the past 5 years but without an ulcer present at the time of enrollment. The dose regimen in the studies was PRILOSEC 20 mg b.i.d. plus clarithromycin 500 mg b.i.d. plus amoxicillin 1 g b.i.d. for 10 days; or clarithromycin 500 mg b.i.d. plus amoxicillin 1 g b.i.d. for 10 days. In studies 126 and 127, patients who took the omeprazole regimen also received an additional 18 days of PRILOSEC 20 mg q.d. Endpoints studied were eradication of *H. pylori* and duodenal ulcer healing (studies 126 and 127 only). *H. pylori* status was determined by CLOtest®, histology and culture in all three studies. For a given patient, *H. pylori* was considered eradicated if at least two of these tests were negative, and none was positive.

The combination of omeprazole plus clarithromycin plus amoxicillin was effective in eradicating *H. pylori*.

[See first table at top of next page]

Dual Therapy (PRILOSEC/clarithromycin)—Four randomized, double-blind, multi-center studies (M93-067, M93-100, M92-812b, and M93-058) evaluated PRILOSEC 40 mg q.d. plus clarithromycin 500 mg t.i.d. for 14 days, followed by

PRILOSEC 20 mg q.d. (M93-067, M93-100, M93-058) or by PRILOSEC 40 mg q.d. (M92-812b) for an additional 14 days in patients with active duodenal ulcer associated with *H. pylori*. Studies M93-067 and M93-100 were conducted in the U.S. and Canada and enrolled 242 and 256 patients, respectively. *H. pylori* infection and duodenal ulcer were confirmed in 219 patients in Study M93-067 and 228 patients in Study M93-100. These studies compared the combination regimen to PRILOSEC and clarithromycin monotherapies. Studies M92-812b and M93-058 were conducted in Europe and enrolled 154 and 215 patients, respectively. *H. pylori* infection and duodenal ulcer were confirmed in 148 patients in study M92-812b and 208 patients in Study M93-058. These studies compared the combination regimen to omeprazole monotherapy. The results for the efficacy analyses for these studies are described below. *H. pylori* eradication was defined as no positive test (culture or histology) at 4 weeks following the end of treatment, and two negative tests were required to be considered eradicated of *H. pylori*. In the per-protocol analysis, the following patients were excluded: dropouts, patients with missing *H. pylori* tests post-treatment, and patients that were not assessed for *H. pylori* eradication because they were found to have an ulcer at the end of treatment.

The combination of omeprazole and clarithromycin was effective in eradicating *H. pylori*.

[See second table above]

Ulcer healing was not significantly different when clarithromycin was added to omeprazole therapy compared to omeprazole therapy alone.

The combination of omeprazole and clarithromycin was effective in eradicating *H. pylori* and reduced duodenal ulcer recurrence.

[See third table above]

Gastric Ulcer

In a U.S. multicenter, double-blind, study of omeprazole 40 mg once a day, 20 mg once a day, and placebo in 520 patients with endoscopically diagnosed gastric ulcer, the following results were obtained.

Treatment of Gastric Ulcer
% of Patients Healed
(All Patients Treated)

	PRILOSEC 20 mg q.d. (n = 202)	PRILOSEC 40 mg q.d. (n = 214)	Placebo (n = 104)
Week 4	47.5**	55.6**	30.8
Week 8	74.8**	82.7**,+	48.1

**(p < 0.01) PRILOSEC 40 mg or 20 mg versus placebo
+(p < 0.05) PRILOSEC 40 mg versus 20 mg

For the stratified groups of patients with ulcer size less than or equal to 1 cm, no difference in healing rates between 40 mg and 20 mg was detected at either 4 or 8 weeks. For patients with ulcer size greater than 1 cm, 40 mg was significantly more effective than 20 mg at 8 weeks.

In a foreign, multinational, double-blind study of 602 patients with endoscopically diagnosed gastric ulcer, omeprazole 40 mg once a day, 20 mg once a day, and ranitidine 150 mg twice a day were evaluated.

Treatment of Gastric Ulcer
% of Patients Healed
(All Patients Treated)

	PRILOSEC 20 mg q.d. (n = 200)	PRILOSEC 40 mg q.d. (n = 187)	Ranitidine 150 mg b.i.d. (n = 199)
Week 4	63.5	78.1**,++	56.3
Week 8	81.5	91.4**,++	78.4

**(p < 0.01) PRILOSEC 40 mg versus ranitidine
++(p < 0.01) PRILOSEC 40 mg versus 20 mg

Gastroesophageal Reflux Disease (GERD)

Symptomatic GERD

A placebo controlled study was conducted in Scandinavia to compare the efficacy of omeprazole 20 mg or 10 mg once daily for up to 4 weeks in the treatment of heartburn and other symptoms in GERD patients without erosive esophagitis. Results are shown below.

% Successful Symptomatic Outcome[a]

	PRILOSEC 20 mg a.m.	PRILOSEC 10 mg a.m.	Placebo a.m.
All patients	46*,† (n = 205)	31† (n = 199)	13 (n = 105)
Patients with confirmed GERD	56*,† (n = 115)	36† (n = 109)	14 (n = 59)

[a]Defined as complete resolution of heartburn
*(p < 0.005) versus 10 mg
†(p < 0.005) versus placebo

Erosive Esophagitis

In a U.S. multicenter double-blind placebo controlled study of 20 mg or 40 mg of PRILOSEC Delayed-Release Capsules in patients with symptoms of GERD and endoscopically diagnosed erosive esophagitis of grade 2 or above, the percentage healing rates (per protocol) were as follows:

Week	20 mg PRILOSEC (n = 83)	40 mg PRILOSEC (n = 87)	Placebo (n = 43)
4	39**	45**	7
8	74**	75**	14

**(p < 0.01) PRILOSEC versus placebo.

In this study, the 40 mg dose was not superior to the 20 mg dose of PRILOSEC in the percentage healing rate. Other controlled clinical trials have also shown that PRILOSEC is effective in severe GERD. In comparisons with histamine H_2-receptor antagonists in patients with erosive esophagitis, grade 2 or above, PRILOSEC in a dose of 20 mg was significantly more effective than the active controls. Complete daytime and nighttime heartburn relief occurred significantly faster (p < 0.01) in patients treated with PRILOSEC than in those taking placebo or histamine H_2-receptor antagonists.

In this and five other controlled GERD studies, significantly more patients taking 20 mg omeprazole (84%) reported complete relief of GERD symptoms than patients receiving placebo (12%).

Long Term Maintenance Treatment of Erosive Esophagitis

In a U.S. double-blind, randomized, multicenter, placebo controlled study, two dose regimens of PRILOSEC were studied in patients with endoscopically confirmed healed esophagitis. Results to determine maintenance of healing of erosive esophagitis are shown below.

Life Table Analysis

	PRILOSEC 20 mg q.d. (n = 138)	PRILOSEC 20 mg 3 days per week (n = 137)	Placebo (n = 131)
Percent in endoscopic remission at 6 months	*70	34	11

*(p < 0.01) PRILOSEC 20 mg q.d. versus PRILOSEC 20 mg 3 consecutive days per week or placebo.

In an international multicenter double-blind study, PRILOSEC 20 mg daily and 10 mg daily were compared to ranitidine 150 mg twice daily in patients with endoscopically confirmed healed esophagitis. The table below provides the results of this study for maintenance of healing of erosive esophagitis.

Life Table Analysis

	PRILOSEC 20 mg q.d. (n = 131)	PRILOSEC 10 mg q.d. (n = 133)	Ranitidine 150 mg b.i.d. (n = 128)
Percent in endoscopic remission at 12 months	*77	‡58	46

*(p = 0.01) PRILOSEC 20 mg q.d. versus PRILOSEC 10 mg q.d. or Ranitidine.
‡(p = 0.03) PRILOSEC 10 mg q.d. versus Ranitidine.

In patients who initially had grades 3 or 4 erosive esophagitis, for maintenance after healing 20 mg daily of PRILOSEC was effective, while 10 mg did not demonstrate effectiveness.

Pathological Hypersecretory Conditions

In open studies of 136 patients with pathological hypersecretory conditions, such as Zollinger-Ellison (ZE) syndrome with or without multiple endocrine adenomas, PRILOSEC Delayed-Release Capsules significantly inhibited gastric acid secretion and controlled associated symptoms of diar-

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Per-Protocol and Intent-to-Treat *H. pylori* Eradication Rates
% of Patients Cured [95% Confidence Interval]

	PRILOSEC +clarithromycin +amoxicillin		Clarithromycin +amoxicillin	
	Per-Protocol†	Intent-to-Treat‡	Per-Protocol†	Intent-to-Treat‡
Study 126	*77 [64, 86] (n = 64)	*69 [57, 79] (n = 80)	43 [31, 56] (n = 67)	37 [27, 48] (n = 84)
Study 127	*78 [67, 88] (n = 65)	*73 [61, 82] (n = 77)	41 [29, 54] (n = 68)	36 [26, 47] (n = 83)
Study M96-446	*90 [80, 96] (n = 69)	*83 [74, 91] (n = 84)	33 [24, 44] (n = 93)	32 [23, 42] (n = 99)

†Patients were included in the analysis if they had confirmed duodenal ulcer disease (active ulcer, studies 126 and 127; history of ulcer within 5 years, study M96-446) and *H. pylori* infection at baseline defined as at least two of three positive endoscopic tests from CLOtest®, histology, and/or culture. Patients were included in the analysis if they completed the study. Additionally, if patients dropped out of the study due to an adverse event related to the study drug, they were included in the analysis as failures of therapy. The impact of eradication on ulcer recurrence has not been assessed in patients with a past history of ulcer.

‡Patients were included in the analysis if they had documented *H. pylori* infection at baseline and had confirmed duodenal ulcer disease. All dropouts were included as failures of therapy.

*(p < 0.05) versus clarithromycin plus amoxicillin.

H. pylori Eradication Rates (Per-Protocol Analysis at 4 to 6 Weeks)
% of Patients Cured [95% Confidence Interval]

	PRILOSEC + Clarithromycin	PRILOSEC	Clarithromycin
U.S. Studies			
Study M93-067	74 [60, 85]†‡ (n = 53)	0 [0, 7] (n = 54)	31 [18, 47] (n = 42)
Study M93-100	64 [51, 76]†‡ (n = 61)	0 [0, 6] (n = 59)	39 [24, 55] (n = 44)
Non U.S. Studies			
Study M92-812b	83 [71, 92]‡ (n = 60)	1 [0, 7] (n = 74)	N/A
Study M93-058	74 [64, 83]‡ (n = 86)	1 [0, 6] (n = 90)	N/A

†Statistically significantly higher than clarithromycin monotherapy (p < 0.05)
‡Statistically significantly higher than omeprazole monotherapy (p < 0.05)

Duodenal Ulcer Recurrence Rates by *H. pylori* Eradication Status
% of Patients with Ulcer Recurrence

	H. pylori eradicated[#]	*H. pylori* not eradicated[#]
U.S. Studies†		
6 months post-treatment		
Study M93-067	*35 (n = 49)	60 (n = 88)
Study M93-100	*8 (n = 53)	60 (n = 106)
Non U.S. Studies‡		
6 months post-treatment		
Study M92-812b	*5 (n = 43)	46 (n = 78)
Study M93-058	*6 (n = 53)	43 (n = 107)
12 months post-treatment		
Study M92-812b	*5 (n = 39)	68 (n = 71)

[#] *H. pylori* eradication status assessed at same timepoint as ulcer recurrence
†Combined results for PRILOSEC + clarithromycin, PRILOSEC, and clarithromycin treatment arms
‡Combined results for PRILOSEC + clarithromycin and PRILOSEC treatment arms
*(p ≤ 0.01) versus proportion with duodenal ulcer recurrence who were not *H. pylori* eradicated

Prilosec—Cont.

rhea, anorexia, and pain. Doses ranging from 20 mg every other day to 360 mg per day maintained basal acid secretion below 10 mEq/hr in patients without prior gastric surgery, and below 5 mEq/hr in patients with prior gastric surgery. Initial doses were titrated to the individual patient need, and adjustments were necessary with time in some patients (see DOSAGE AND ADMINISTRATION). PRILOSEC was well tolerated at these high dose levels for prolonged periods (> 5 years in some patients). In most ZE patients, serum gastrin levels were not modified by PRILOSEC. However, in some patients serum gastrin increased to levels greater than those present prior to initiation of omeprazole therapy. At least 11 patients with ZE syndrome on long-term treatment with PRILOSEC developed gastric carcinoids. These findings are believed to be a manifestation of the underlying condition, which is known to be associated with such tumors, rather than the result of the administration of PRILOSEC (see ADVERSE REACTIONS).

Microbiology

Omeprazole and clarithromycin dual therapy and omeprazole, clarithromycin and amoxicillin triple therapy have been shown to be active against most strains of *Helicobacter pylori in vitro* and in clinical infections as described in the INDICATIONS AND USAGE section.

Helicobacter

Helicobacter pylori

Pretreatment Resistance

Clarithromycin pretreatment resistance rates were 3.5% (4/113) in the omeprazole/clarithromycin dual therapy studies (M93-067, M93-100) and 9.3% (41/439) in omeprazole/clarithromycin/amoxicillin triple therapy studies (126, 127, M96-446).

Amoxicillin pretreatment susceptible isolates (≤ 0.25 µg/mL) were found in 99.3% (436/439) of the patients in the omeprazole/clarithromycin/amoxicillin triple therapy studies (126, 127, M96-446). Amoxicillin pretreatment minimum inhibitory concentrations (MICs) > 0.25 µg/mL occurred in 0.7% (3/439) of the patients, all of whom were in the clarithromycin and amoxicillin study arm. One patient had an unconfirmed pretreatment amoxicillin minimum inhibitory concentration (MIC) of > 256 µg/mL by Etest®.

[See table below]

Patients not eradicated of *H. pylori* following omeprazole/clarithromycin/amoxicillin triple therapy or omeprazole/clarithromycin dual therapy will likely have clarithromycin resistant *H. pylori* isolates. Therefore, clarithromycin susceptibility testing should be done, if possible. Patients with clarithromycin resistant *H. pylori* should not be treated with any of the following: omeprazole/clarithromycin dual therapy, omeprazole/clarithromycin/amoxicillin triple therapy, or other regimens which include clarithromycin as the sole antimicrobial agent.

Amoxicillin Susceptibility Test Results and Clinical/Bacteriological Outcomes

In the triple therapy clinical trials, 84.9% (157/185) of the patients in the omeprazole/clarithromycin/amoxicillin treatment group who had pretreatment amoxicillin susceptible MICs (≤ 0.25 µg/mL) were eradicated of *H. pylori* and 15.1% (28/185) failed therapy. Of the 28 patients who failed triple therapy, 11 had no post-treatment susceptibility test results and 17 had post-treatment *H. pylori* isolates with amoxicillin susceptible MICs. Eleven of the patients who failed triple therapy also had post-treatment *H. pylori* isolates with clarithromycin resistant MICs.

Susceptibility Test for *Helicobacter pylori*

The reference methodology for susceptibility testing of *H. pylori* is agar dilution MICs[1]. One to three microliters of an inoculum equivalent to a No. 2 McFarland standard ($1 \times 10^7 - 1 \times 10^8$ CFU/mL for *H. pylori*) are inoculated directly onto freshly prepared antimicrobial containing Mueller-Hinton agar plates with 5% aged defibrinated sheep blood (≥ 2 weeks old). The agar dilution plates are incubated at 35°C in a microaerobic environment produced by a gas generating system suitable for campylobacters. After 3 days of incubation, the MICs are recorded as the lowest concentration of antimicrobial agent required to inhibit growth of the organism. The clarithromycin and amoxicillin MIC values should be interpreted according to the following criteria:

Clarithromycin MIC (µg/mL)[a]	Interpretation
≤ 0.25	Susceptible (S)
0.5	Intermediate (I)
≥ 1.0	Resistant (R)
Amoxicillin MIC (µg/mL)[a,b]	**Interpretation**
≤ 0.25	Susceptible (S)

[a] These are tentative breakpoints for the agar dilution methodology and they should not be used to interpret results obtained using alternative methods.

[b] There were not enough organisms with MICs > 0.25 µg/mL to determine a resistance breakpoint.

Standardized susceptibility test procedures require the use of laboratory control microorganisms to control the technical aspects of the laboratory procedures. Standard clarithromycin and amoxicillin powders should provide the following MIC values:

Microorganism	Antimicrobial Agent	MIC (µg/mL)[a]
H. pylori ATCC 43504	Clarithromycin	0.016–0.12 (µg/mL)
H. pylori ATCC 43504	Amoxicillin	0.016–0.12 (µg/mL)

[a] These are quality control ranges for the agar dilution methodology and they should not be used to control test results obtained using alternative methods.

INDICATIONS AND USAGE

Duodenal Ulcer

PRILOSEC Delayed-Release Capsules are indicated for short-term treatment of active duodenal ulcer. Most patients heal within four weeks. Some patients may require an additional four weeks of therapy.

PRILOSEC Delayed-Release Capsules, in combination with clarithromycin and amoxicillin, are indicated for treatment of patients with *H. pylori* infection and duodenal ulcer disease (active or up to 1-year history) to eradicate *H. pylori*.

PRILOSEC Delayed-Release Capsules, in combination with clarithromycin, are indicated for treatment of patients with *H. pylori* infection and duodenal ulcer disease to eradicate *H. pylori*.

Eradication of *H. pylori* has been shown to reduce the risk of duodenal ulcer recurrence (see CLINICAL PHARMACOLOGY, Clinical Studies and DOSAGE AND ADMINISTRATION).

Among patients who fail therapy, PRILOSEC with clarithromycin is more likely to be associated with the development of clarithromycin resistance as compared with triple therapy. In patients who fail therapy, susceptibility testing should be done. If resistance to clarithromycin is demonstrated or susceptibility testing is not possible, alternative antimicrobial therapy should be instituted. (See Microbiology section, and the clarithromycin package insert, MICROBIOLOGY section.)

Gastric Ulcer

PRILOSEC Delayed-Release Capsules are indicated for short-term treatment (4–8 weeks) of active benign gastric ulcer (see CLINICAL PHARMACOLOGY, Clinical Studies, Gastric Ulcer).

Treatment of Gastroesophageal Reflux Disease (GERD)

Symptomatic GERD

PRILOSEC Delayed-Release Capsules are indicated for the treatment of heartburn and other symptoms associated with GERD.

Erosive Esophagitis

PRILOSEC Delayed-Release Capsules are indicated for the short-term treatment (4–8 weeks) of erosive esophagitis which has been diagnosed by endoscopy (see CLINICAL PHARMACOLOGY, Clinical Studies).

The efficacy of PRILOSEC used for longer than 8 weeks in these patients has not been established. In the rare instance of a patient not responding to 8 weeks of treatment, it may be helpful to give up to an additional 4 weeks of treatment. If there is recurrence of erosive esophagitis or GERD symptoms (eg, heartburn), additional 4–8 week courses of omeprazole may be considered.

Maintenance of Healing of Erosive Esophagitis

PRILOSEC Delayed-Release Capsules are indicated to maintain healing of erosive esophagitis.

Controlled studies do not extend beyond 12 months.

Pathological Hypersecretory Conditions

PRILOSEC Delayed-Release Capsules are indicated for the long-term treatment of pathological hypersecretory conditions (eg, Zollinger-Ellison syndrome, multiple endocrine adenomas and systemic mastocytosis).

CONTRAINDICATIONS

Omeprazole

PRILOSEC Delayed-Release Capsules are contraindicated in patients with known hypersensitivity to any component of the formulation.

Clarithromycin

Clarithromycin is contraindicated in patients with a known hypersensitivity to any macrolide antibiotic.

Concomitant administration of clarithromycin with cisapride, pimozide, or terfenadine is contraindicated. There have been post-marketing reports of drug interactions when clarithromycin and/or erythromycin are co-administered with cisapride, pimozide, or terfenadine resulting in cardiac arrhythmias (QT prolongation, ventricular tachycardia, ventricular fibrillation, and torsades de pointes) most likely due to inhibition of hepatic metabolism of these drugs by erythromycin and clarithromycin. Fatalities have been reported. (Please refer to full prescribing information for clarithromycin before prescribing.)

Amoxicillin

Amoxicillin is contraindicated in patients with a history of allergic reaction to any of the penicillins. (Please refer to full prescribing information for amoxicillin before prescribing.)

WARNINGS

Clarithromycin

CLARITHROMYCIN SHOULD NOT BE USED IN PREGNANT WOMEN EXCEPT IN CLINICAL CIRCUMSTANCES WHERE NO ALTERNATIVE THERAPY IS APPROPRIATE. IF PREGNANCY OCCURS WHILE TAKING CLARITHROMYCIN, THE PATIENT SHOULD BE APPRISED OF THE POTENTIAL HAZARD TO THE FETUS. (See WARNINGS in prescribing information for clarithromycin.)

Amoxicillin

SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (anaphylactic) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE LIKELY TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR A HISTORY OF SENSITIVITY TO MULTIPLE ALLERGENS. BEFORE INITIATING THERAPY WITH AMOXICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS OR OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, AMOXICILLIN SHOULD BE DISCONTINUED AND APPROPRIATE THERAPY INSTITUTED. **SERIOUS ANAPHYLACTIC REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.** (See WARNINGS in prescribing information for amoxicillin.)

Antimicrobials

Pseudomembranous colitis has been reported with nearly all antibacterial agents and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents. (See WARNINGS in prescribing information for clarithromycin and amoxicillin.)

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an antibacterial drug clinically effective against *Clostridium difficile* colitis.

PRECAUTIONS

General

Symptomatic response to therapy with omeprazole does not preclude the presence of gastric malignancy.

Clarithromycin Susceptibility Test Results and Clinical/Bacteriological Outcomes

Clarithromycin Susceptibility Test Results and Clinical/Bacteriological Outcomes[a]

Clarithromycin Pretreatment Results		Clarithromycin Post-treatment Results				
		H. pylori negative - eradicated	*H. pylori* positive - not eradicated Post-treatment susceptibility results			
			S[b]	I[b]	R[b]	No MIC
Dual Therapy - (omeprazole 40 mg q.d./clarithromycin 500 mg t.i.d. for 14 days followed by omeprazole 20 mg q.d. for another 14 days) (Studies M93-067, M93-100)						
Susceptible[b]	108	72	1		26	9
Intermediate[b]	1				1	
Resistant[b]	4				4	
Triple Therapy - (omeprazole 20 mg b.i.d./clarithromycin 500 mg b.i.d./amoxicillin 1 g b.i.d. for 10 days - Studies 126, 127, M96-446; followed by omeprazole 20 mg q.d. for another 18 days - Studies 126, 127)						
Susceptible[b]	171	153	7		3	8
Intermediate[b]						
Resistant[b]	14	4	1		6	3

[a] Includes only patients with pretreatment clarithromycin susceptibility test results

[b] Susceptible (S) MIC ≤ 0.25 µg/mL, Intermediate (I) MIC 0.5 – 1.0 µg/mL, Resistant (R) MIC ≥ 2 µg/mL

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Atrophic gastritis has been noted occasionally in gastric corpus biopsies from patients treated long-term with omeprazole.

Information for Patients

PRILOSEC Delayed-Release Capsules should be taken before eating. Patients should be cautioned that the PRILOSEC Delayed-Release Capsule should not be opened, chewed or crushed, and should be swallowed whole.

For patients who have difficulty swallowing capsules, the contents of a PRILOSEC Delayed-Release Capsule can be added to applesauce. One tablespoon of applesauce should be added to an empty bowl and the capsule should be opened. All of the pellets inside the capsule should be carefully emptied on the applesauce. The pellets should be mixed with the applesauce and then swallowed immediately with a glass of cool water to ensure complete swallowing of the pellets. The applesauce used should not be hot and should be soft enough to be swallowed without chewing. The pellets should not be chewed or crushed. The pellets/applesauce mixture should not be stored for future use.

Drug Interactions

Other

Omeprazole can prolong the elimination of diazepam, warfarin and phenytoin, drugs that are metabolized by oxidation in the liver. Although in normal subjects no interaction with theophylline or propranolol was found, there have been clinical reports of interaction with other drugs metabolized via the cytochrome P-450 system (eg, cyclosporine, disulfiram, benzodiazepines). Patients should be monitored to determine if it is necessary to adjust the dosage of these drugs when taken concomitantly with PRILOSEC.

Because of its profound and long lasting inhibition of gastric acid secretion, it is theoretically possible that omeprazole may interfere with absorption of drugs where gastric pH is an important determinant of their bioavailability (eg, ketoconazole, ampicillin esters, and iron salts). In the clinical trials, antacids were used concomitantly with the administration of PRILOSEC.

Combination Therapy with Clarithromycin

Co-administration of omeprazole and clarithromycin have resulted in increases in plasma levels of omeprazole, clarithromycin, and 14-hydroxy-clarithromycin (see also CLINICAL PHARMACOLOGY, Pharmacokinetics: Combination Therapy with Antimicrobials).

Concomitant administration of clarithromycin with cisapride, pimozide, or terfenadine is contraindicated.

There have been reports of an interaction between erythromycin and astemizole resulting in QT prolongation and torsades de pointes. Concomitant administration of erythromycin and astemizole is contraindicated. Because clarithromycin is also metabolized by cytochrome P450, concomitant administration of clarithromycin with astemizole is not recommended. (see also CONTRAINDICATIONS, Clarithromycin, above. Please refer to full prescribing information for clarithromycin before prescribing).

Carcinogenesis, Mutagenesis, Impairment of Fertility

In two 24-month carcinogenicity studies in rats, omeprazole at daily doses of 1.7, 3.4, 13.8, 44.0 and 140.8 mg/kg/day (approximately 4 to 352 times the human dose, based on a patient weight of 50 kg and a human dose of 20 mg) produced gastric ECL cell carcinoids in a dose-related manner in both male and female rats; the incidence of this effect was markedly higher in female rats, which had higher blood levels of omeprazole. Gastric carcinoids seldom occur in the untreated rat. In addition, ECL cell hyperplasia was present in all treated groups of both sexes. In one of these studies, female rats were treated with 13.8 mg omeprazole/kg/day (approximately 35 times the human dose) for one year, then followed for an additional year without the drug. No carcinoids were seen in these rats. An increased incidence of treatment-related ECL cell hyperplasia was observed at the end of one year (94% treated vs 10% controls). By the second year the difference between treated and control rats was much smaller (46% vs 26%) but still showed more hyperplasia in the treated group. An unusual primary malignant tumor in the stomach was seen in one rat (2%). No similar tumor was seen in male or female rats treated for two years. For this strain of rat no similar tumor has been noted historically, but a finding involving only one tumor is difficult to interpret. A 78-week mouse carcinogenicity study of omeprazole did not show increased tumor occurrence, but the study was not conclusive. A 26-week p53+/- transgenic mouse carcinogenicity study was not positive.

Omeprazole was not mutagenic in an *in vitro* Ames *Salmonella typhimurium* assay, an *in vitro* mouse lymphoma cell assay and an *in vivo* rat liver DNA damage assay. A mouse micronucleus test at 625 and 6250 times the human dose gave a borderline result, as did an *in vivo* bone marrow chromosome aberration test. A second mouse micronucleus study at 2000 times the human dose, but with different (suboptimal) sampling times, was negative.

In a rat fertility and general reproductive performance test, omeprazole in a dose range of 13.8 to 138.0 mg/kg/day (approximately 35 to 345 times the human dose) was not toxic or deleterious to the reproductive performance of parental animals.

Pregnancy

Omeprazole

Pregnancy Category C

Teratology studies conducted in pregnant rats at doses up to 138 mg/kg/day (approximately 345 times the human dose) and in pregnant rabbits at doses up to 69 mg/kg/day (approximately 172 times the human dose) did not disclose any evidence for a teratogenic potential of omeprazole.

In rabbits, omeprazole in a dose range of 6.9 to 69.1 mg/kg/day (approximately 17 to 172 times the human dose) produced dose-related increases in embryo-lethality, fetal resorptions and pregnancy disruptions. In rats, dose-related embryo/fetal toxicity and postnatal developmental toxicity were observed in offspring resulting from parents treated with omeprazole 13.8 to 138.0 mg/kg/day (approximately 35 to 345 times the human dose). There are no adequate or well-controlled studies in pregnant women. Sporadic reports have been received of congenital abnormalities occurring in infants born to women who have received omeprazole during pregnancy. Omeprazole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Clarithromycin

Pregnancy Category C. See WARNINGS (above) and full prescribing information for clarithromycin before using in pregnant women.

Nursing Mothers

It is not known whether omeprazole is excreted in human milk. In rats, omeprazole administration during late gestation and lactation at doses of 13.8 to 138 mg/kg/day (35 to 345 times the human dose) resulted in decreased weight gain in pups. Because many drugs are excreted in human milk, because of the potential for serious adverse reactions in nursing infants from omeprazole, and because of the potential for tumorigenicity shown for omeprazole in rat carcinogenicity studies, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use

The safety and effectiveness of PRILOSEC have been established in the age group 2 years to 16 years for the treatment of acid-related gastrointestinal diseases, including the treatment of symptomatic GERD, treatment of erosive esophagitis, and the maintenance of healing of erosive esophagitis. The safety and effectiveness of PRILOSEC have not been established for pediatric patients less than 2 years of age. Use of PRILOSEC in the age group 2 years to 16 years is supported by evidence from adequate and well-controlled studies of PRILOSEC in adults with additional clinical, pharmacokinetic, and safety studies performed in pediatric patients (see CLINICAL PHARMACOLOGY, Pharmacokinetics and Metabolism: Omeprazole).

Treatment of Gastroesophageal Reflux Disease (GERD)

Symptomatic GERD

In an uncontrolled, open-label study of patients aged 2 years to 16 years with a history of symptoms suggestive of nonerosive GERD, 113 patients were assigned to receive a single daily dose of omeprazole (10 mg or 20 mg, based on body weight) either as an intact capsule or as an open capsule in applesauce. Results showed success rates of 60% (10 mg omeprazole) and 59% (20 mg omeprazole) in reducing the number and intensity of either pain-related symptoms or vomiting/regurgitation episodes.

Erosive Esophagitis

In an uncontrolled, open-label dose-titration study, healing of erosive esophagitis in pediatric patients aged 1 to 16 years required doses that ranged from 0.7 to 3.5 mg/kg/day (80 mg/day). Doses were initiated at 0.7 mg/kg/day. Doses were increased in increments of 0.7 mg/kg/day (if intra-esophageal pH showed a pH of < 4 for less than 6% of a 24-hour study). After titration, patients remained on treatment for 3 months. Forty-four percent of the patients were healed on a dose of 0.7 mg/kg body weight; most of the remaining patients were healed with 1.4 mg/kg after an additional 3 months' treatment. Erosive esophagitis was healed in 51 of 57 (90%) children who completed the first course of treatment in the healing phase of the study. In addition, after 3 months of treatment, 33% of the children had no overall symptoms, 57% had mild reflux symptoms, and 40% had less frequent regurgitation/vomiting.

Maintenance of Healing of Erosive Esophagitis

In an uncontrolled, open-label study of maintenance of healing of erosive esophagitis in 46 pediatric patients, 54% of patients required half the healing dose. The remaining patients increased the healing dose (0.7 to a maximum of 2.8 mg/kg/day) either for the entire maintenance period, or returned to half the dose before completion. Of the 46 patients who entered the maintenance phase, 19 (41%) had no relapse. In addition, maintenance therapy in erosive esophagitis patients resulted in 63% of patients having no overall symptoms.

Safety

The safety of PRILOSEC Delayed-Release Capsules has been assessed in 310 pediatric patients aged 0 to 16 years and 62 physiologically normal volunteers aged 2 years to 16 years. Of the 310 pediatric patients with acid-related disease, a group of 46 who had documented healing of erosive esophagitis after 3 months of treatment continued on maintenance therapy for up to 749 days.

PRILOSEC Delayed-Release Capsules administered to pediatric patients was generally well tolerated with an adverse event profile resembling that in adults. Unique to the pediatric population, however, adverse events of the respiratory system were most frequently reported in both the 0 to 2 year and 2 to 16 year age groups (46.2% and 18.5%, respectively). Similarly, otitis media was frequently reported in the 0 to 2 year age group (22.6%), and accidental injuries were reported frequently in the 2 to 16 year age group (3.8%).

Geriatric Use

Omeprazole was administered to over 2000 elderly individuals (≥ 65 years of age) in clinical trials in the US and Europe. There were no differences in safety and effectiveness between the elderly and younger subjects. Other reported clinical experience has not identified differences in response between the elderly and younger subjects, but greater sensitivity of some older individuals cannot be ruled out.

Pharmacokinetic studies have shown the elimination rate was somewhat decreased in the elderly and bioavailability was increased. The plasma clearance of omeprazole was 250 mL/min (about half that of young volunteers) and its plasma half-life averaged one hour, about twice that of young healthy volunteers. However, no dosage adjustment is necessary in the elderly (see CLINICAL PHARMACOLOGY).

ADVERSE REACTIONS

PRILOSEC Delayed-Release Capsules were generally well tolerated during domestic and international clinical trials in 3096 patients.

In the U.S. clinical trial population of 465 patients (including duodenal ulcer, Zollinger-Ellison syndrome and resistant ulcer patients), the following adverse experiences were reported to occur in 1% or more of patients on therapy with PRILOSEC. Numbers in parentheses indicate percentages of the adverse experiences considered by investigators as possibly, probably or definitely related to the drug:

	Omeprazole (n = 465)	Placebo (n = 64)	Ranitidine (n = 195)
Headache	6.9 (2.4)	6.3	7.7 (2.6)
Diarrhea	3.0 (1.9)	3.1 (1.6)	2.1 (0.5)
Abdominal Pain	2.4 (0.4)	3.1	2.1
Nausea	2.2 (0.9)	3.1	4.1 (0.5)
URI	1.9	1.6	2.6
Dizziness	1.5 (0.6)	0.0	2.6 (1.0)
Vomiting	1.5 (0.4)	4.7	1.5 (0.5)
Rash	1.5 (1.1)	0.0	0.0
Constipation	1.1 (0.9)	0.0	0.0
Cough	1.1	0.0	1.5
Asthenia	1.1 (0.2)	1.6 (1.6)	1.5 (1.0)
Back Pain	1.1	0.0	0.5

The following adverse reactions which occurred in 1% or more of omeprazole-treated patients have been reported in international double-blind, and open-label, clinical trials in which 2,631 patients and subjects received omeprazole.

Incidence of Adverse Experiences ≥ 1%
Causal Relationship not Assessed

	Omeprazole (n = 2631)	Placebo (n = 120)
Body as a Whole, site unspecified		
Abdominal pain	5.2	3.3
Asthenia	1.3	0.8
Digestive System		
Constipation	1.5	0.8
Diarrhea	3.7	2.5
Flatulence	2.7	5.8
Nausea	4.0	6.7
Vomiting	3.2	10.0
Acid regurgitation	1.9	3.3
Nervous System / Psychiatric		
Headache	2.9	2.5

Additional adverse experiences occurring in < 1% of patients or subjects in domestic and/or international trials, or occurring since the drug was marketed, are shown below within each body system. In many instances, the relationship to PRILOSEC was unclear.

Body as a Whole: Allergic reactions, including, rarely, anaphylaxis (see also *Skin* below), fever, pain, fatigue, malaise, abdominal swelling

Cardiovascular: Chest pain or angina, tachycardia, bradycardia, palpitation, elevated blood pressure, peripheral edema

Gastrointestinal: Pancreatitis (some fatal), anorexia, irritable colon, flatulence, fecal discoloration, esophageal candidiasis, mucosal atrophy of the tongue, dry mouth. During treatment with omeprazole, gastric fundic gland polyps have been noted rarely. These polyps are benign and appear to be reversible when treatment is discontinued.

Gastro-duodenal carcinoids have been reported in patients with ZE syndrome on long-term treatment with PRILOSEC. This finding is believed to be a manifestation of the underlying condition, which is known to be associated with such tumors.

Hepatic: Mild and, rarely, marked elevations of liver function tests [ALT (SGPT), AST (SGOT), γ-glutamyl transpeptidase, alkaline phosphatase, and bilirubin (jaundice)]. In rare instances, overt liver disease has occurred, including hepatocellular, cholestatic, or mixed hepatitis, liver necrosis (some fatal), hepatic failure (some fatal), and hepatic encephalopathy.

Metabolic / Nutritional: Hyponatremia, hypoglycemia, weight gain

Musculoskeletal: Muscle cramps, myalgia, muscle weakness, joint pain, leg pain

Nervous System / Psychiatric: Psychic disturbances including depression, aggression, hallucinations, confusion, insomnia, nervousness, tremors, apathy, somnolence, anxiety, dream abnormalities; vertigo; paresthesia; hemifacial dysesthesia

Continued on next page

Prilosec—Cont.

Respiratory: Epistaxis, pharyngeal pain
Skin: Rash and, rarely, cases of severe generalized skin reactions including toxic epidermal necrolysis (TEN; some fatal), Stevens-Johnson syndrome, and erythema multiforme (some severe); purpura and/or petechiae (some with rechallenge); skin inflammation, urticaria, angioedema, pruritus, alopecia, dry skin, hyperhidrosis
Special Senses: Tinnitus, taste perversion
Urogenital: Interstitial nephritis (some with positive rechallenge), urinary tract infection, microscopic pyuria, urinary frequency, elevated serum creatinine, proteinuria, hematuria, glycosuria, testicular pain, gynecomastia
Hematologic: Rare instances of pancytopenia, agranulocytosis (some fatal), thrombocytopenia, neutropenia, anemia, leucocytosis, and hemolytic anemia have been reported.
The incidence of clinical adverse experiences in patients greater than 65 years of age was similar to that in patients 65 years of age or less.

Combination Therapy for *H. pylori* Eradication

In clinical trials using either dual therapy with PRILOSEC and clarithromycin, or triple therapy with PRILOSEC, clarithromycin, and amoxicillin, no adverse experiences peculiar to these drug combinations have been observed. Adverse experiences that have occurred have been limited to those that have been previously reported with omeprazole, clarithromycin, or amoxicillin.
Triple Therapy (PRILOSEC/clarithromycin/amoxicillin) — The most frequent adverse experiences observed in clinical trials using combination therapy with PRILOSEC, clarithromycin, and amoxicillin (n = 274) were diarrhea (14%), taste perversion (10%), and headache (7%). None of these occurred at a higher frequency than that reported by patients taking the antimicrobial drugs alone.
For more information on clarithromycin or amoxicillin, refer to the respective package inserts, ADVERSE REACTIONS sections.
Dual Therapy (PRILOSEC/clarithromycin) — Adverse experiences observed in controlled clinical trials using combination therapy with PRILOSEC and clarithromycin (n = 346) which differed from those previously described for omeprazole alone were: Taste perversion (15%), tongue discoloration (2%), rhinitis (2%), pharyngitis (1%) and flu syndrome (1%).
For more information on clarithromycin, refer to the clarithromycin package insert, ADVERSE REACTIONS section.

OVERDOSAGE

Reports have been received of overdosage with omeprazole in humans. Doses ranged up to 2400 mg (120 times the usual recommended clinical dose). Manifestations were variable, but included confusion, drowsiness, blurred vision, tachycardia, nausea, vomiting, diaphoresis, flushing, headache, dry mouth, and other adverse reactions similar to those seen in normal clinical experience (see ADVERSE REACTIONS). Symptoms were transient, and no serious clinical outcome has been reported when PRILOSEC was taken alone. No specific antidote for omeprazole overdosage is known. Omeprazole is extensively protein bound and is, therefore, not readily dialyzable. In the event of overdosage, treatment should be symptomatic and supportive.
As with the management of any overdose, the possibility of multiple drug ingestion should be considered. For current information on treatment of any drug overdose, a certified Regional Poison Control Center should be contacted. Telephone numbers are listed in the Physicians' Desk Reference (PDR) or local telephone book.
Single oral doses of omeprazole at 1350, 1339, and 1200 mg/kg were lethal to mice, rats, and dogs, respectively. Animals given these doses showed sedation, ptosis, tremors, convulsions, and decreased activity, body temperature, and respiratory rate and increased depth of respiration.

DOSAGE AND ADMINISTRATION

Short-Term Treatment of Active Duodenal Ulcer

The recommended adult oral dose of PRILOSEC is 20 mg once daily. Most patients heal within four weeks. Some patients may require an additional four weeks of therapy (see INDICATIONS AND USAGE).

***H. pylori* Eradication for the Reduction of the Risk of Duodenal Ulcer Recurrence**

Triple Therapy (PRILOSEC/clarithromycin/amoxicillin) — The recommended adult oral regimen is PRILOSEC 20 mg plus clarithromycin 500 mg plus amoxicillin 1000 mg each given twice daily for 10 days. In patients with an ulcer present at the time of initiation of therapy, an additional 18 days of PRILOSEC 20 mg once daily is recommended for ulcer healing and symptom relief.
Dual Therapy (PRILOSEC/clarithromycin) — The recommended adult oral regimen is PRILOSEC 40 mg once daily plus clarithromycin 500 mg t.i.d. for 14 days. In patients with an ulcer present at the time of initiation of therapy, an additional 14 days of PRILOSEC 20 mg once daily is recommended for ulcer healing and symptom relief.
Please refer to clarithromycin full prescribing information for CONTRAINDICATIONS and WARNINGS, and for information regarding dosing in elderly and renally impaired patients (see PRECAUTIONS: General, PRECAUTIONS: Geriatric Use and PRECAUTIONS: Drug Interactions).
Please refer to amoxicillin full prescribing information for CONTRAINDICATIONS and WARNINGS.

Gastric Ulcer

The recommended adult oral dose is 40 mg once a day for 4–8 weeks (see CLINICAL PHARMACOLOGY, Clinical Studies, Gastric Ulcer, and INDICATIONS AND USAGE, Gastric Ulcer).

Gastroesophageal Reflux Disease (GERD)

The recommended adult oral dose for the treatment of patients with symptomatic GERD and no esophageal lesions is 20 mg daily for up to 4 weeks. The recommended adult oral dose for the treatment of patients with erosive esophagitis and accompanying symptoms due to GERD is 20 mg daily for 4 to 8 weeks (see INDICATIONS AND USAGE).

Maintenance of Healing of Erosive Esophagitis

The recommended adult oral dose is 20 mg daily (see CLINICAL PHARMACOLOGY, Clinical Studies).

Pathological Hypersecretory Conditions

The dosage of PRILOSEC in patients with pathological hypersecretory conditions varies with the individual patient. The recommended adult oral starting dose is 60 mg once a day. Doses should be adjusted to individual patient needs and should continue for as long as clinically indicated. Doses up to 120 mg t.i.d. have been administered. Daily dosages of greater than 80 mg should be administered in divided doses. Some patients with Zollinger-Ellison syndrome have been treated continuously with PRILOSEC for more than 5 years.

Pediatric Patients

For the treatment of GERD or other acid-related disorders, the recommended dose for pediatric patients 2 years of age and older is as follows:

Patient Weight	Omeprazole Dose
< 20 kg	10 mg
≥ 20 kg	20 mg

On a per kg basis, the doses of omeprazole required to heal erosive esophagitis are greater than those for adults.
For pediatric patients unable to swallow an intact capsule, see Alternative Administration Options subsection below.

Alternative Administration Options

For patients who have difficulty swallowing capsules, the contents of a PRILOSEC Delayed-Release Capsule can be added to applesauce. One tablespoon of applesauce should be added to an empty bowl and the capsule should be opened. All of the pellets inside the capsule should be carefully emptied on the applesauce. The pellets should be mixed with the applesauce and then swallowed immediately with a glass of cool water to ensure complete swallowing of the pellets. The applesauce used should not be hot and should be soft enough to be swallowed without chewing. The pellets should not be chewed or crushed. The pellets/applesauce mixture should not be stored for future use.
No dosage adjustment is necessary for patients with renal impairment or for the elderly.
PRILOSEC Delayed-Release Capsules should be taken before eating. In the clinical trials, antacids were used concomitantly with PRILOSEC.
Patients should be cautioned that the PRILOSEC Delayed-Release Capsule should not be opened, chewed or crushed, and should be swallowed whole.

HOW SUPPLIED

No. 3426 — PRILOSEC Delayed-Release Capsules, 10 mg, are opaque, hard gelatin, apricot and amethyst colored capsules, coded 606 on cap and PRILOSEC 10 on the body. They are supplied as follows:
NDC 0186-0606-31 unit of use bottles of 30
NDC 0186-0606-68 bottles of 100
NDC 0186-0606-28 unit dose packages of 100
NDC 0186-0606-82 bottles of 1000.
No. 3440 — PRILOSEC Delayed-Release Capsules, 20 mg, are opaque, hard gelatin, amethyst colored capsules, coded 742 on cap and PRILOSEC 20 on body. They are supplied as follows:
NDC 0186-0742-31 unit of use bottles of 30
NDC 0186-0742-28 unit dose package of 100
NDC 0186-0742-82 bottles of 1000.
No. 3428 — PRILOSEC Delayed-Release Capsules, 40 mg, are opaque, hard gelatin, apricot and amethyst colored capsules, coded 743 on cap and PRILOSEC 40 on the body. They are supplied as follows:
NDC 0186-0743-31 unit of use bottles of 30
NDC 0186-0743-68 bottles of 100
NDC 0186-0743-28 unit dose packages of 100
NDC 0186-0743-82 bottles of 1000.

Storage

Store PRILOSEC Delayed-Release Capsules in a tight container protected from light and moisture. Store between 15°C and 30°C (59°F and 86°F).

REFERENCES

1. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fifth Edition. Approved Standard NCCLS Document M7-A5, Vol, 20, No. 2, NCCLS, Wayne, PA, January 2000.

Manufactured for: AstraZeneca LP, Wilmington, DE 19850
By: Merck & Co., Inc., Whitehouse Station, NJ 08889, USA

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Shown in Product Identification Guide, page 305

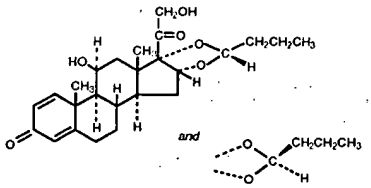
PULMICORT RESPULES® ℞

{pûl-mĭ-cōrt}
(budesonide inhalation suspension)
0.25 mg and 0.5 mg
℞ only

For inhalation use via compressed air driven jet nebulizers only (not for use with ultrasonic devices). Not for injection. Read patient instructions before using.

DESCRIPTION

Budesonide, the active component of PULMICORT RESPULES®, is a corticosteroid designated chemically as (RS)-11β, 16α, 17, 21-tetrahydroxypregna-1, 4-diene-3, 20-dione cyclic 16, 17-acetal with butyraldehyde. Budesonide is provided as a mixture of two epimers (22R and 22S). The empirical formula of budesonide is $C_{25}H_{34}O_6$ and its molecular weight is 430.5. Its structural formula is:



Budesonide is a white to off-white, tasteless, odorless powder that is practically insoluble in water and in heptane, sparingly soluble in ethanol, and freely soluble in chloroform. Its partition coefficient between octanol and water at pH 7.4 is 1.6×10^3.
PULMICORT RESPULES is a sterile suspension for inhalation via jet nebulizer and contains the active ingredient budesonide (micronized), and the inactive ingredients disodium edetate, sodium chloride, sodium citrate, citric acid, polysorbate 80, and Water for Injection. Two dose strengths are available in single-dose ampules (Respules™ ampules): 0.25 mg and 0.5 mg per 2 mL RESPULE ampule.
For PULMICORT RESPULES, like all other nebulized treatments, the amount delivered to the lungs will depend on patient factors, the jet nebulizer utilized, and compressor performance. Using the Pari-LC-Jet Plus Nebulizer/Pari Master compressor system, under *in vitro* conditions, the mean delivered dose at the mouthpiece (% nominal dose) was approximately 17% at a mean flow rate of 5.5 L/min. The mean nebulization time was 5 minutes or less.
PULMICORT RESPULES should be administered from jet nebulizers at adequate flow rates, via face masks or mouthpieces (see DOSAGE AND ADMINISTRATION).

CLINICAL PHARMACOLOGY

Mechanism of Action

Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity and weak mineralocorticoid activity. In standard *in vitro* and animal models, budesonide has approximately a 200-fold higher affinity for the glucocorticoid receptor and a 1000-fold higher topical anti-inflammatory potency than cortisol (rat croton oil ear edema assay). As a measure of systemic activity, budesonide is 40 times more potent than cortisol when administered subcutaneously and 25 times more potent when administered orally in the rat thymus involution assay.
The precise mechanism of corticosteroid actions on inflammation in asthma is not well known. Corticosteroids have been shown to have a wide range of inhibitory activities against multiple cell types (eg, mast cells, eosinophils, neutrophils, macrophages, and lymphocytes) and mediators (eg, histamine, eicosanoids, leukotrienes, and cytokines) involved in allergic- and non-allergic-mediated inflammation. The anti-inflammatory actions of corticosteroids may contribute to their efficacy in asthma.
Studies in asthmatic patients have shown a favorable ratio between topical anti-inflammatory activities and systemic corticosteroid effects over a wide dose range of inhaled budesonide in a variety of formulations and delivery systems including Pulmicort Turbuhaler® (an inhalation-driven, multi-dose dry powder inhaler) and the inhalation suspension for nebulization. This is explained by a combination of a relatively high local anti-inflammatory effect, extensive first pass hepatic degradation of orally absorbed drug (85–95%) and the low potency of metabolites (see below).

Pharmacokinetics

The activity of PULMICORT RESPULES is due to the parent drug, budesonide. In glucocorticoid receptor affinity studies, the 22R form was two times as active as the 22S epimer. *In vitro* studies indicated that the two forms of budesonide do not interconvert.
Budesonide is primarily cleared by the liver. In asthmatic children 4–6 years of age, the terminal half-life of budesonide after nebulization is 2.3 hours, and the systemic clearance is 0.5 L/min, which is approximately 50% greater than in healthy adults after adjustment for differences in weight.
After a single dose of 1 mg budesonide, a peak plasma concentration of 2.6 nmol/L was obtained approximately 20

XI. Related Proceedings Appendix to Appeal Brief Under Rule 41.37(c)(1)(x)

No related proceeding decisions are known to Appellant, Appellant's legal representative, or the Assignee.